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# *The anatomical record*

Charles Russell Bardeen, Irving Hardesty, Association of American Anatomists, John Lewis Bremer, American Association of Anatomists, ...

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# THE PHYLOGENETIC RELATIONS OF THE LYMPHATIC AND BLOOD VASCULAR SYSTEMS IN VERTEBRATES

GEO. S. HUNTINGTON

*From the Anatomical Laboratory of Columbia University*

At a time when the ontogenesis of the vertebrate, and especially of the mammalian, lymphatic system has called forth general interest and considerable activity in research, it seems advisable to regard the mutual relations of the hæmal and lymphatic divisions of the vertebrate vascular system from the standpoint of their phylogeny, in as far as material for such observations is to date available, and in turn to fit the facts ascertained by the study of mammalian lymphatic ontogenesis into the framework obtained by these generalized comparisons.

This appears all the more advisable when we consider that any valid theory of lymphatic development must, on the one hand, agree in its postulates with the phylogenetic facts, as far as they are definitely established, and that, on the other hand, a review of the ascertained comparative conditions of the two systems in their mutual bearing on the general question will serve to direct attention to the problems of vascular morphology as yet imperfectly known, and thus guide the inquiries in the right direction.

In the earliest zoölogical conditions in multicellular organisms a simple circulation suffices to supply all the metabolic demands of the tissues. Such a circulation is attained when a system of intercellular canals develops in which a clear plasmatic fluid, without free cellular elements moves in response to the pulsations of contractile areas included within the system.

In general terms a circulation of this kind is, from the phylogenetic standpoint, a primary formation and represents the primitive lymphatic type of vascular organization, from which in higher forms the hæmal system develops as a secondary vascular

acquisition. In the physiological sense this establishment of a hæmal circulation, as a graft on the earlier circulation of non-cellular plasma, is to be interpreted as the expression of the increased rate of tissue combustion and the resulting growing demands of the organism for oxygen, required in the ascent to higher animal types. The biochemical agent which makes this elevation from the lower to the next higher rung of the zoölogical ladder possible, is the hæmoglobin, and the morphological expression of its employment is found in the genesis of the modified mesodermal cell entering the circulation as the free red blood cell.

The first circulation of this type contains in the plasma-stream cells which enable the organism to establish a very simple type of respiration. Such a condition in its earliest inception is represented by the circulatory apparatus of *Amphioxus*. With the elevation to more advanced vertebrate organization this mixed lymphatico-hæmal type of circulation no longer suffices for the growing respiratory requirements, and, in response to the resulting functional demand, a separation of the original single circulatory system into two divisions begins to develop. One of these divisions continues on the hereditary lines as the rudiment of the future lymphatic system in the narrower sense, with cell free plasma, while the other, arising primarily in response to the ever increasing demand of the tissues for oxygen, differentiates as the anlage of a hæmal circulation with the hæmoglobin cell as its distinctive biochemical and morphological character.

In this process the bloodvascular system has gradually and continuously assumed more and more complex relations to the organism and has taken on successively higher and higher biochemical activities in addition to the original function of serving in the respiratory exchange, until it has morphologically and physiologically become the predominant vascular structure. The separation from the lymphatic channels has in consequence become more and more pronounced, until it has progressed in mammals to the point where, as the final outcome of the process, two distinct sets of vessels are established side by side. The component channels of one set, further specialized as arteries,

veins, blood capillaries and heart, are closely associated and mutually interdependent, and give structural expression to the importance attained in the course of phylogenetic evolution by the hæmal vascular system. The second set, that of the lymphatic channels, is in its main extent independent of the first, simpler and more primitive in its organization, and more restricted in function, closely interlocked with the venous division of the blood-vascular system which it in certain functional respects supplements.

This division must of course have occurred gradually, and rather by multiplication of channels than by septal division of a single preëxisting system into two components. Naturally this process should offer in the phylogenetic series many stages in which the division is still incomplete, and hence the gradual solution of peripheral organic continuity between hæmal and lymphatic vascular organization, in proceeding from the lower to the higher types, is, even with our present scanty knowledge, quite evident in the vertebrate series. No recent contributions to comparative vascular anatomy have done more to clear this question and to advance our correct perspective than the investigations of Favaro<sup>1</sup> on vascular organization in fishes.

The Italian anatomist has described a very close and intimate relationship in teleosts between lymphatic and venous organization. In several forms the same vessels appear to function at certain periods as lymphatic channels, while at others they are physiologically venous in character. Hand in hand with this interchangeable functional relationship goes a marked complexity of the lymphatic and venous "hearts."

The investigations of Allen<sup>2</sup> have led to similar results.

In ascending the zoölogical scale we encounter, together with the increasing independence of hæmal and lymphatic vascular

<sup>1</sup> Favaro, Guiseppe, 1905: Il cuore ed i seni caudali dei Teleostei Ahi R, 1. Veneto di sc. lettr et arti, 1905-06, tome 65- Part II. Append. alla Dispensa, bet. 1906, Venejin 1906. Anat. Anz. Bd. 27, p. 879-880. Archivio di Fisiologia, Bd. 2, Fasc. 5.

<sup>2</sup> Allen, W. F.: Distribution of the Subcutaneous Vessels in the Head Region of the Ganoids, Polydon and Lepisosteus. Washington Acad. Sc. Proc., vol. ix, pp. 79-188, 1907. Distribution of the Subcutaneous Vessels in the Tail Region of Lepisosteus. Am. Jour. Anatomy, vol. viii, pp. 50-89, 1908.

organization, a progressive and phylogenetically most significant reduction in the number, complexity and histological differentiation of the lymph hearts. Favaro's demonstration of the primitive relationship between the teleostean lymphatic and venous system now completes the chain between the conditions presented by *Amphioxus* and those encountered next above the fishes in the urodele amphibians. This ascent is marked by a more definite separation of venous and lymphatic pathways, although the still large number (14-20) of the urodele veno-lymphatic hearts recalls the former more intimate association of the two systems.

A reduction of the lymph hearts to two pairs, an anterior and a posterior, is next encountered in the anure amphibians. In the reptiles only a single (posterior) pair of these organs is carried into the adult organization. The anterior lymph heart appears, however, during the ontogenesis in a rudimentary form, as determined by recent examinations of the 7.5 mm. and 9 mm. embryo of *Scleroporus undulatus*.

In birds the posterior lymph heart is retained throughout life in some forms. In others, as shown by Sala,<sup>3</sup> it develops in the embryo, but disappears soon after the assumption of free life. While the subject is still under investigation, there is reason to believe that the presence of a rudimentary anterior lymph heart in birds during the ontogenesis can be demonstrated, and that this structure effects the final lymphatico-venous connection.

In mammals, finally, a reduced single pair of anterior organs, the jugular lymph sacs, alone persists, as far as we at present know, as the sole remnants of the extensive antecedent series of lymphatico-venous connections and serves as the bond between the otherwise completely separated venous and lymphatic systems.

Thus the more prominent and highly specialized the hæmal vascular system appears as compared with the lymphatic, the higher in general is the animal organization possessing this type. Conversely, in descending the zoölogical series, the individuality and independence of the two systems diminishes steadily until they finally merge into the single common archæal anlage.

<sup>3</sup> Sala, L.: Sullo sviluppo dei cuori linfatici e dei dotti toracici nell' embrione di pollo. Roma, 1900.

As previously stated<sup>4</sup> the lymph hearts represent in this evolutionary process, on the one hand, the line along which the gradually acquired organic separation of the blood-vascular and lymphatic systems proceeds, while on the other hand, they play in the various phases of this process of segmentation the important rôle of links between the lymphatic and the hæmal channels, which, in ascending the scale, are becoming progressively and increasingly more independent of each other.

For obvious physiological and mechanical reasons this original connection can never be entirely interrupted, but we see in the relative organization of the lymphatic and venous systems in the mammalia, in comparison with the conditions formed in lower vertebrates, the highest degrees of this phylogenetically acquired independence.

I have in a previous publication<sup>5</sup> pointed out the fact that the possibility exists in the plan of mammalian lymphatic organization for the establishment of lymphatico-venous connections at other points in the adult than those afforded by the typical connections of the anterior lymph heart or jugular lymph sac with the veins at the common jugular and jugulo-subclavian angles. Such connections, if they exist in certain forms, must be interpreted with our present knowledge of mammalian lymphatic organization, as retentions of other primitive lymph heart bonds between the lymphatic and venous system which, in the greater number of mammalia, are not developed and carried into the typical adult plan, but which may, while atypical for the general class, appear in certain specialized forms.

It is quite conceivable that development on these lines would lead to results which would serve the mammalian physiological demands equally well, if not better, than the prevalent type of mammalian lymphatic organization. It is possible that the reported instances of the termination of the thoracic duct in one of the azygos veins or its tributaries in the adult human subject can be interpreted as variations depending for their genesis on the

<sup>4</sup> Huntington: The Genetic Interpretation of the Development of the Mammalian Lymphatic System. *Anatomical Record*, vol. ii, nos. 1 and 2, 1908, pp. 1-44.

<sup>5</sup> Loc. cit., p. 30.



atypical development and retention of a lymphatico-venous heart formation at points other than the ones normally concerned in the production of the jugular lymph sac. The reported cases are, however, not sufficiently authentic to accept them at their apparent face value, and the available evidence is too scanty to warrant the assumption that these variations, if they exist, are of a progressive character tending toward the eventual reduction of the thoracic duct and the substitution for the same of a more direct connection of the abdominal lymphatic channels with the venous system.

The more our knowledge of comparative vascular anatomy grows, the clearer the perception becomes, that in spite of the apparent structural and functional differences between venous and lymphatic organization, the two systems are but parts of an originally single and united whole, and hence must be primarily of equal and identical origin. The genetic unity of all vascular structure is a proposition which is constantly becoming more self-evident. Even if it were not for the direct observations to the contrary, this fact alone negatives the assumption of the derivation of the systemic lymphatics from the veins as secondary products of their endothelial proliferation. The line of reasoning above outlined, if carried to its logical conclusion, stamps the entire complicated hæmal vascular apparatus of the higher vertebrate types as the genetic descendant of a pre-existing simple lymphatic vascular system. In other words, in place of considering our modern lymphatics as derivatives from the veins, I believe that in a correct valuation of the relative position of veins and lymphatics, we are obliged to regard the lymphatic system as the primary organization, from which gradually in the phylogenesis the bloodvascular system has been derived. In spite of the predominance of hæmal over lymphatic structure in the higher forms, the latter should be recognized as the phylogenetically older primary structure.

The separation between the two has in the evolutionary sense become more and more pronounced, until it has progressed in the mammal to the point where, even in the ontogenesis, the anlagen of both are laid down independently. But their common genetic

basis is to be found in the vascular strands of the early mesoderm. It is however not at all improbable that the mammalian lymphatic system, as we at present know it, in the relatively few forms that have been carefully studied, is still in the evolutionary sense undergoing progressive changes which in their broader significance trend toward further reduction and simplification of the lymphatic, as compared with the hæmal vascular organization. In view of the predominant association of the mammalian lymphatic channels with intestinal alimentation and the metabolic processes of digestion, such further evolutionary modification of lymphatic organization from the type now prevalent in mammalia, would in all probability be in the direction of still greater development of this physiological character. This might find structural expression in the higher development of the intestinal lymphatic complex and a coincident reduction of the general lymphatic channels at present associated with them. The mammalian lymphatic system would under these conditions correspond mainly to the hepatic-portal venous channels and would convey the products of digestion directly to the systemic venous current.

The organic principle of the above described phylogenetic separation of a hæmal from a preëxisting single primitive lymphatic circulation repeats itself within the far narrower circle of the former in the phylogenetic division which leads, through the Dipnoean and Perennibranchiate lines, to the replacement of the primitive single branchial type of respiration and circulation by the double cycle of the air breathing forms. This change in environment, with its resulting enlarged scope of vertebrate life, has led step by step to more highly organized structural types within the framework of the primitive hæmal vascular system, through which stages the single-hearted, cold-blooded branchial form has advanced to the double-hearted, warm-blooded pulmonary type.

In its physiological significance this general evolutionary process again means primarily vastly greater and more rapid tissue metabolism or combustion in the broad sense. The structural response to this functional demand is strikingly given in the

phylogenetic (and ontogenetic) development of the intra-cardiac and intra-aortic septa. We encounter here on a large and unmistakable scale, and associated with an evident biochemical function, the division of part of the originally simple and uniform hæmal vascular system of cardiac chambers and truncus arteriosus into two bilateral and equivalent elements. This change is effected primarily not by addition from *without* (except the neomorphism of the pulmonary vein) of something *new* but by a change and rearrangement of parts already existing *within* the framework of the primary bloodvascular system. If, therefore, as McClure and I have definitely proved,<sup>\*</sup> the mammalian jugular lymph sac, or cervical lymph heart, is secondarily separated from the mammalian embryonic pre- and postcardinal veins, this process of division of originally single hæmal channels into completely separate elements is not genetically a *new* process, confined to the lymphatico-venous terminal, but follows on a smaller scale and much more obscurely, genetic lines already laid down in the division of the primitive single heart tube into its completely distinct dextral and sinistral components.

The question here involved is of great and far reaching importance in establishing the correct relative position and value of the hæmal and lymphatic vascular systems.

If the metabolic demand for increased and more rapid supply of oxygen is capable of calling into existence, within the already organized confines of the hæmal division of a simple vertebrate circulation, the structural changes leading to the divided heart and the lung in place of the antecedent branchial type of circulation and respiration, then the same force is evidently sufficient to derive, in far earlier phylogenetic stages, from the primitive general non-cellular circulatory system, a separate set of channels conveying plasma with free hæmoglobin cells as the circulating medium, and developed primarily in the service of the oxygen-carbon dioxide exchange of the tissues.

In this way there comes to be established the phylogenetic

<sup>\*</sup> Huntington and McClure: The Anatomy and Development of the Jugular Lymph Sacs in the Domestic Cat. *Anatomical Record*, vol. ii, nos. 1-2, 1908, pp. 1-18.

anlage of a secondary bloodvascular system, derived from the primitive general vascular apparatus circulating non-cellular plasma. With the appearance of the hæmal system the distinction between it and the persistent portion of the primitive vascular organization as a lymphatic system develops.

Thus the primitive simple hæmal system, subsequently destined to undergo under the stimulus of phylogenetic advance, a complete secondary division, was in its own turn originally segmented from a simpler antecedent circulation of lymphatic type for the purpose of satisfying the earliest demand of the tissues for oxygen by becoming the carrier of hæmoglobin cells, while the persistent elements of the earlier system are retained as lymphatic vessels serving a new physiological purpose under changed conditions of metabolism.

As stated above the series of lymph hearts would in this genesis of the bloodvascular system represent points where the original continuity of lymphatic and hæmal elements is retained, in a specialized and modified form for definite physiological purposes. The number and distinctive character of these lymph hearts would then naturally diminish in proceeding serially from the lowest to the highest types, coincident with the serially developed greater and greater independence of the hæmal and lymphatic divisions of a general vascular system.

This change implies an enormous degree of adaptability and structural response to functional demands. Many examples of this extreme plasticity of vascular organization are encountered throughout the entire formative period of the mammalian embryo, in which the bloodvascular system is the predominant agency of nutrition as well as respiration. This character appears not only in the crystallization of definite assymetrical arterial and venous pathways from an antecedent symetrical bilateral type, but also in many of the more intricate relations of the bloodvascular channels to the temporary and the future permanent metabolic demands of the tissues. Thus, for instance, in the placentalia the vitelline veins appear in the rôle of the earliest embryonic nutritive and respiratory channels. They subsequently, in the placental period, yield this part to the secondarily involved um-

bilicals. Their own primary significance is lost and remains in abeyance throughout the whole of the placental epoch, to suddenly reassert itself when the hepatic portal channels, as the direct descendants of the afferent vitelline veins, assume with the establishment of intestinal alimentation at birth, the important share in the nutritive processes of the body which they are henceforth to maintain throughout the life of the individual. The anlagen of these vessels were, so to speak, side-tracked for the very considerable umbilical or placental period of embryonic and foetal existence. But they continued to develop during this entire period of functional displacement and obscurity, and became associated with the growing alimentary canal, in anticipation of the moment when, with the first establishment of post-foetal conditions, they resumed their original significance and entered into their now definite and permanent function as nutrient afferent hepatic vessels.

In the same way the entire extensive series of structural changes within the three divisions of the bloodvascular system, leading finally to the establishment of the pulmonary circulation, is developed in anticipation of the sudden assumption of pulmonary respiration at birth.

This law of anticipatory ontogenesis is of very wide application and expresses especially the cardinal character of extreme adaptability, both to present requirements and future needs of the organism, in all developing vascular structure.

It is quite possible, that the lymphatic vessels, which we must recognize in the broad phylogenetic ground plan of vascular organization, as the primary and earliest channels, appear in the complicated and highly specialized mammalian vascular system of predominantly hæmal type, in a subordinate and secondary position, owing to genetic influences of this general character.

They are formed, during the embryonic period, just as the portal and pulmonary channels are formed, but like these, they develop in anticipation of assuming their functional activity only with the altered environment and changed nutritive conditions of the post-foetal period.

In this sense they appear as secondary structures, allied to the

all important hæmal embryonic channels, just as the placental vitelline veins, within narrower phylogenetic limits, appear subordinate to the new bloodvascular conditions dependent upon the acquisition of the umbilical vein as the main embryonic nutritive and respiratory vessel, in the phylogenetic ascent from the vitelline to the placental phase of embryonic development.

This brings us to the question of the comparison between the ontogenesis of the mammalian systemic lymphatics and the lymphatic organization of the lower vertebrates. Briefly stated, our observation as to the development of the mammalian lymphatic vessels, can be summed up as follows:

1. The first anlagen of the bloodvascular channels and of the systemic lymphatic vessels in the mammalian embryo are identical. These common anlagen are formed by independent intercellular mesodermal tissue spaces, which, in enlarging, become lined, in obedience to the mechanical pressure effects of the clear fluid contents of the spaces, with endothelium.

2. The spaces become confluent to form larger and continuous channels. The bloodvascular system differentiates genetically from the lymphatic system by the secondary inclusion of the specialized mesodermal hæmoglobin cell of the blood islands in the clear non-cellular stream of the plasma circulating during the primary stage in the hæmal system of channels in response to the cardiac pulsations.

The systemic lymphatic channels continue on the other hand, to convey a clear fluid containing no, or only a few, cellular elements.

3. This histogenetic identity, and the fact that subsequently the only criterion defining respectively the early embryonic bloodvascular and lymphatic channels is the red blood cell content of the former, precludes definite differentiation of the two sets of vessels prior to the period at which the hæmal channels acquire their distinctive free cellular elements.

4. Hence we must accept three chronological possibilities in regard to the ontogenetic period at which these anlagen begin to appear.:

- a. The bloodvascular and lymphatic channels develop simul-



taneously as capillary anlagen side by side, and subsequently differentiate from each other as above detailed.

b. The lymphatic anlagen are the first to develop. Subsequently a portion of the common system, or a second generation of equivalent channels, differentiates as the hæmal component of the vascular system, in contradistinction to the persisting primary lymphatic system.

c. The bloodvascular system is ontogenetically the first to develop in the mammalian embryo. The lymphatic anlagen appear secondarily as an equivalent system of mesodermal spaces, which subsequently unite. The resulting channel system does not acquire the free circulating blood cells characteristic of the hæmal division, but finally gains access to the blood vascular system by union with the jugular lymph sacs, derived from the veins, and is thus enabled to enter as an integral component into the triple constitution of the general circulatory apparatus.

From the phylogenetic standpoint the second of the above enumerated possibilities is the one which is most consistent with the hæmo-lymphatic organization as seen in its general evolution in the vertebrate series. At the same time the last of the three possibilities appears from the evidence at hand to represent most accurately the conditions encountered in mammalian embryos. The separation between bloodvascular and systemic lymphatic organizations has here not only progressed to a degree in which even the ontogenetic anlagen of the two channel systems are laid down independently of each other, but has further resulted in placing their first appearance into different embryonic periods.

From the phylogenetic standpoint this must be regarded as the result of factors operative in the specialization of the highest vertebrate types, and not as the original common condition. The mammalian ontogenetic relationship between the hæmal and lymphatic anlagen appears as an expression of the tremendous development which in the evolution of the higher zoological types, the bloodvascular system has gained over the primary lymphatic circulation. This paramount influence of hæmal over lymphatic vascular development has even reversed the relative ontogenetic period in which the first distinct anlagen of

each system appear. The bloodvascular organization has gained the complete ascendancy, the lymphatic has been relegated to a secondary position, with highly curtailed and specialized function. Moreover, as above stated, the actual assumption of this function has been in the mammalian ontogenesis postponed to the end of the placental epoch, and the assumption of individual nutrition with the establishment of the definite postnatal conditions.

Compared with the position of the lymphatic circulation in the ancestral series, one is almost tempted to characterize its development in the placental embryo as the reversional appearance of a system, formerly of much greater extent and importance, but now to a large extent replaced by more modern zoölogical acquisitions, and retained only in a modified and reduced form with greatly restricted functional application.

At any rate, there is no radical inconsistency in the observed facts, either of the phylogenetic or ontogenetic history of vertebrate lymphatic vessels.

In respect to their genesis in the mammalian embryo, it makes but little difference as to exact embryonic period in which they make their first appearance as definite lymphatic anlagen.

Their development may be synchronous with that of the earliest hæmal channels, or precede these, or finally, as seems to be actually the case, they may first appear distinctly after the main embryonic bloodvascular lines have been laid down.

Their ultimate secondary union with each other, and then with the venous system through the intervention of the complicated jugular lymph sacs, and the entire character of the completed adult lymphatic-system as a "shadow-picture" of the venous organization, suggests strongly that the mammalian lymphatic vessels have phylogenetically acquired this secondary position relative to the dominant hæmal vascular system.

This subordination of lymphatic to bloodvascular structures manifests itself not only in the morphological relations existing ontogenetically and in the adult between the two systems, but the same influence has operated to retard the embryonic appearance of the first definite lymphatic anlagen to a period in which the

blood-vascular organization has already assumed clear cut and definite character.

We thus reach the end-link in the long chain of successive differentiations which lead through the vertebrate series to the final stage in which the greatest attainable degree of independence between lymphatic and hæmal vascular structure has been reached, and in which the primitive relative value to the organism of the two systems has been reversed, in obedience to the law which has stamped the bloodvascular system as the main organic line of evolutionary progress.

## PANCREATIC BLADDERS

WILLIAM SNOW MILLER

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For several years I have not given the course in mammalian anatomy which forms part of the work introductory to the study of human anatomy, at the University of Wisconsin. During the summer session of the present year (1909) it fell again to my lot to give this course, and one of the animals used presented a variation not unusual in our laboratory, a pancreatic bladder.

In two earlier communications I have called attention to the occurrence in the domestic cat of a pancreatic bladder which bears a similar relation to the pancreatic ducts that the gall bladder does to the bile ducts.

With the description of the present case there are now on record seven cases in which this peculiarity was present and of the seven cases, five have been found in the anatomical laboratory of the University of Wisconsin. The first case was described by Mayer in 1815, a second by Gage in 1879, three cases by myself in 1904 and a fourth case in 1905.

That five of the seven cases should be found in a small community seems to indicate, either that there exists locally a special breed of cats, or that there has been a lack of careful observation on the part of those instructors whose courses include the dissection of the cat.

It is not improbable, however, that some cases of a pancreatic bladder have been overlooked, having been mistaken for cases in which a double gall bladder was present.

It is my custom when students take up the study of the viscera of the cat, to inject with a different colored starch mass the bile ducts and the pancreatic ducts. This is easily done by making an opening in the duodenum 25 to 30 mm. in length opposite the

entrance of the ductus choledochus, thus exposing the oval opening leading into the ampulla of Vater. With but little difficulty a cannula can be inserted and tied in the ductus choledochus and the injection of the bile ducts and gall bladder completed. It often requires some skill and patience to introduce a second cannula by the side of the first into the ductus pancreaticus; but the technique once acquired, rarely does any difficulty present itself.

By this simple procedure the student is able to follow the gross distribution of the bile and pancreatic ducts and, as I usually inject the arterial system, the systemic veins and the portal system with still different colored masses, the relation which these ducts bear to the blood vascular system is easily made out.

The case I now record was found in a young male cat obtained from a part of the city far removed from that of the preceding cases; therefore it seems quite improbable that this cat was in any way related to the others.

The type is that of the second and third cases that I have described, namely, a duct leading from the duodorsal division of the ductus pancreaticus and terminating in a well-defined bladder situated a little to one side of and dorsal to the gall bladder, which occupies its usual position in the right median lobe of the liver. It differs from the other cases in that there is a triangular flap of pancreatic tissue surrounding the entrance of this duct into the duodorsal division of the ductus pancreaticus. Because of this peculiarity the present case occupies a position between the second case described by Heuer (see below) and the cases previously described by myself. The relations of the various parts are shown in fig. 1.

The question as to the origin of these pancreatic bladders at once presents itself, and in this connection it is interesting to note two variations of the pancreas found by Heuer while studying the arrangement of the pancreatic ducts of the cat.

In the first case (fig. 2) a band of glandular tissue, an outgrowth from the caput, passed cephalad, following the ductus choledochus and cystic duct and partially covering them on their ventral side. It extended to

about the middle of the gall bladder, where it fused with the connective tissue around the latter. It had a duct which passed down its entire length and joined the axial branch of the caput. In the second case (fig. 3) a similar though slightly narrower band of glandular tissue extended from the caput alongside of the ductus choledochus and ductus cysticus. It then continued along the left side of the gall bladder to the posterior (ventral?) part of the liver, where it enlarged into an oval

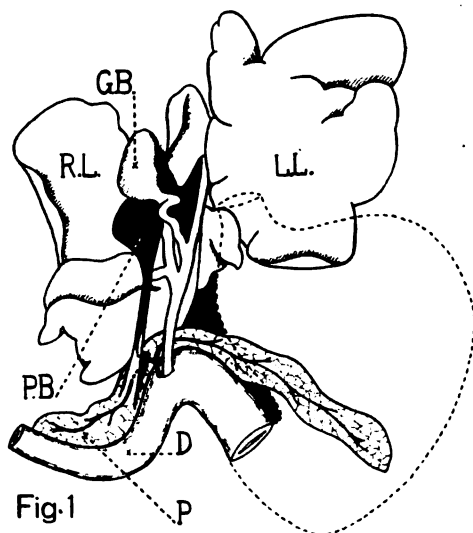


FIG. 1. Drawn in situ. Shows the relation of the pancreas and the pancreatic bladder to the liver, stomach and duodenum. The liver has been turned cephalad; the stomach is in outline.

G. B., gall bladder; R. L., right lateral lobe of the liver; L. L., left lateral lobe of the liver; P. B., pancreatic bladder; P., pancreas; D., duodenum.

The triangular flap of pancreatic tissue mentioned in the text can be seen extending from the pancreas along the duct connected with the pancreatic bladder. One-half the natural size.

nodule about one centimeter in its long diameter. This nodule occupied a hollow in the right central lobe of the liver to the left of the gall bladder. A duct was present which extended from the nodule down the middle of the band, and joined the axial branch of the caput as in the previous case.

The pancreas is usually described as arising from a dorsal and a ventral anlage which fuse after rotation of the duodenum has taken place, the ventral anlage giving rise to the ductus pan-

creaticus (Wirsung) while the ductus accessorius (Santorini) takes its origin from the dorsal anlage.

In cyclostomes and selachians a dorsal anlage only is present, in all the remaining vertebrates a dorsal and ventral anlage is found and the investigations of Stöhr, Göppert, Saint-Remy, Felix, Hammer, Stoss, Wlassow, and others have shown that the ventral anlage is paired, a pancreatic diverticulum arising on

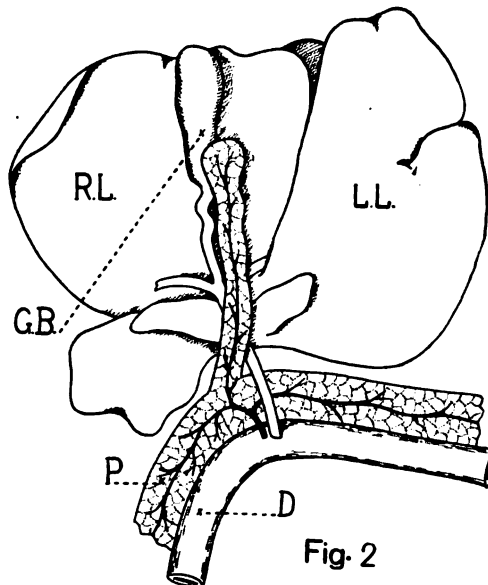


Fig. 2

FIG. 2. Schematic drawing constructed from Heuer's description of his first case of a pancreas with three principal divisions.

D., duodenum; G. B., gall bladder; R. L., right lateral lobe of the liver; L. L., left lateral lobe of the liver; P., pancreas with a band of pancreatic tissue extending along the ductus choledochus.

each side of the liver stalk. The left ventral outgrowth usually disappears, the right persisting and maintaining its association with the liver stalk which now becomes the ductus choledochus.

Returning now to the cases of Heuer: it seems probable that in each case both the right and left ventral anlage persisted, the left in case one (fig. 2) giving rise to the broad band of pancreatic tissue extending along the ductus choledochus and ductus cysti-

cus, while in the second case (fig. 3) the left anlage was drawn out into a long narrow band with an enlarged distal end. Now if we conceive the narrow band of pancreatic tissue in the second of these cases to undergo a degeneration leaving only the duct, and the distal enlarged portion converted into a dilatation, we have a complete series of changes through which these pancreatic bladders may have arisen.

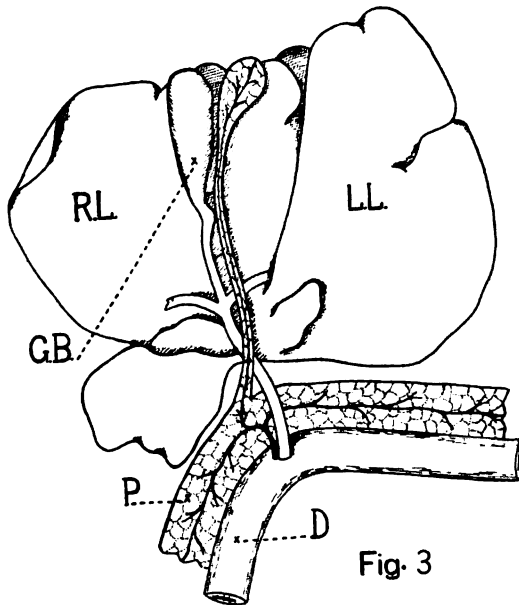


Fig. 3

FIG. 3. Outline drawing of Heuer's second case of a pancreas with three principal divisions.

D., duodenum; G. B., gall bladder; R. L., right lateral lobe of the liver; L. L., left lateral lobe of the liver; P., pancreas with a long narrow band of pancreatic tissue extending along the ductus choledochus and terminating in an expansion situated on the left side of the gall bladder. (After Heuer.)

Another and more probable hypothesis may be advanced, namely: that the ventral anlage, in place of being double, may in these cases be bi-lobed, either from the beginning, or as a result of fusion, one of the lobes having given rise to the caput of the pancreas and the other to the bands of pancreatic tissues found by Heuer, or to the pancreatic bladders which I have found.



That the ventral anlage is in some cases bi-lobed, the investigations of Wlassow on the development of the pancreas of the pig have shown.

I have described this additional case of a pancreatic bladder and suggested two possible explanations of the origin of these bladders in order that attention may again be called to the anomaly and to the embryological questions involved, and to stimulate more careful observation on the part of those who give courses in mammalian anatomy.

#### LITERATURE

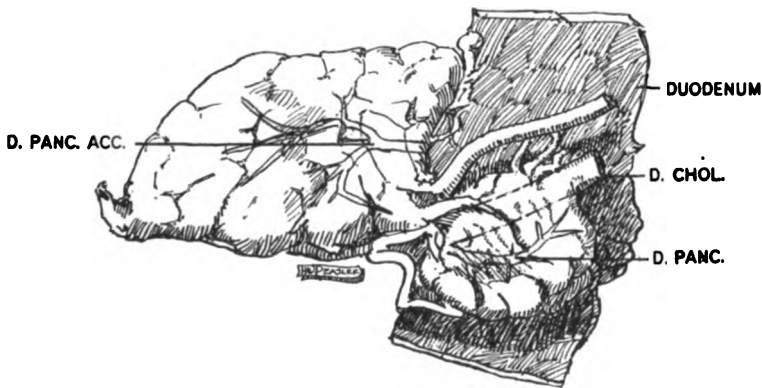
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# AN ADULT HUMAN PANCREAS SHOWING AN EMBRYOLOGICAL CONDITION

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This unusual pancreas was removed from the body of an adult white female, 71 years old, who had died of "valvular heart disease." The abdominal cavity presented a number of anomalous conditions, among these an abnormal duodenum and pancreas. The duodenum, which was of the V-shaped variety, presented an ascending limb lying to the right of and ventral to the descending limb.



The pancreas, represented in the figure as seen from the dorsum, consisted of two parts united with each other by a narrow strand of glandular tissue dorsal to the duodenum. The larger por-

tion lay ventral to the bodies of the first and second lumbard vertebræ upon the left side of the descending limb of the duodenum.

Because of the peculiar V-shape of the duodenum this portion of the pancreas was not situated within the loop nor did it have any relation to the transverse or ascending portions of the duodenum. It lay dorsal to the stomach and extended a distance of only 5.0 cm. towards the left kidney possessing a cephalocaudal diameter of 3 cm. and a maximum thickness of 1.3cm.

Traversing themiddle of the glandular sustance, a single large duct passed with increasing calibre from left to right finally emptying into the duodenum 4.0 cm. caudal to the pylorus.

The other smaller portion of the gland lay dorsal to the duodenum and on a level slightly caudal to the part just described. Its long axis extended along the common bile duct, which traversed it ventrally, a distance of 3.0 cm. with a width of 2.0 cm. and a maximum dorso-ventral dimension of 1.5 cm. Coursing through this tissue a single small duct passed caudally to empty finally upon the dorsal wall of the duodenum in company with the common bile duct. This duct approached the caudal aspect of the latter. The narrow band of pancreatic tissue which joined both portions of the gland was drained by radicles of both ducts, yet the ducts were not in communication with each other. Upon the duodenal mucosa, the openings of the ducts wereseparated by an interval of 3.5 cm., the duct from the larger portion being cephalic and ventral.

This anomaly seems to be an instance of the non-fusion of the primitive ventral and dorsal pancreatic anlages, together with an insufficient "rotation" of the ventral anlage around the duodenum. The dorsal portion of the gland in close apposition to the common bile duct corresponds to the ventral anlage which forms ultimately the caudal portion of the head of the adult pancreas and the terminal portion of the main pancreatic duct. The larger portion, derived from the dorsal anlage, represents the remainder of the head and all of the neck and body of the gland together with the enclosed ducts.

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# THE EARLY HISTOGENESIS OF STRIATED MUSCLE IN THE ŒSOPHAGUS OF THE PIG AND THE DOG- FISH

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WITH TWENTY-FIVE FIGURES

Striated muscle is described by most investigators as coming exclusively from the inner plate of the myotome. In the later development of the tissue each muscle fiber is usually said to arise from a single myoblast. Neither of these statements holds good however, regarding the œsophagus of at least the two forms studied, as will appear from the following description of the early development. A brief review of the literature on this subject will first be given.

## LITERATURE REVIEW

The literature, for convenience, is here divided into three classes: (1) the papers which describe the origin of striated muscle in general from the germ layers; (2) those which describe the transformation of the myoblasts into muscle fibers; and (3) those which describe the histology of the adult œsophageal muscle.

(1) Vertebrate striated muscle, with the exception of one of the inner eye muscles of the chick, is almost universally described as arising from the mesoderm. Herzog ('02) found that in the chick the sphincter of the pupil develops from ectoderm. The mode of transformation of mesoderm into muscle-forming tissue (pre-muscle, as it is termed by Lewis '01) is disputed. The three early derivatives of the mesoderm, the myotome, the mesothelium and the mesenchyme, each have been considered by various writers to be the muscle-forming tissue. For a complete review of the literature on this subject the reader is referred to the paper by Maurer, *Die Entwicklung des Muskelsystems und der elektrischen Organe*,—in the Hertwig's *Handbuch d. Entwicklungs-*

lehre der Wirbeltiere, Bd. 3, (1906). Remak ('55) and Balfour ('85) believed that striated skeletal muscle is derived from both the outer and inner plates of the myotome. The Hertwigs ('81) in Selachians, Minot ('92) in the frog, Godlewski ('02) in the rabbit, mouse and guinea-pig, Dahlgren and Kepner ('08) in *Catostomus*, with many other investigators, found striated muscle arising exclusively from the inner plate of the myotome. The head muscles have usually been described as arising from the epithelium of the head somites (Marshall, '82, in Selachians), but Reuter ('98) found that in the pig the outer eye muscles develop from the head mesenchyme.

Lewis and Bardeen ('01) stated that in the human embryo "The skeletal and muscular structures of the limbs are differentiated from the mesenchyme of the limb buds." Lewis ('01) discovered that in the arm of the human embryo the muscle develops directly from mesenchyme. He gives a good review of the literature on the origin of the limb muscles, to which the reader is referred. Since Lewis' paper appeared, Ingalls ('07) has described the origin of the limb muscles from the outer layer of the myotomes in a 4.9 mm. human embryo.

Mlodowska ('08) studied the development of striated muscles in the chick, mouse, rabbit, and pig, and found that most of the skeletal muscle arises from muscle plates, but that the surrounding mesenchyme aids in the later formation.

(2) There are two general theories advanced to account for the origin of the multinucleated striated muscle fiber from the undifferentiated muscle tissue. One is that each primitive myoblast develops into a single muscle fiber, the other is that several myoblasts fuse to form the muscle fiber. The latter is the syncytial theory of muscle origin. Remak ('50-'55), Kölliker ('51), Schultze ('61), Hertwig-Mark ('92), Minot ('92), Bardeen ('00), Eycleshymer ('04), Maurer ('06), and Dahlgren and Kepner ('08), adhere to the unicellular origin of the muscle fiber. Mayo ('62), Calberla ('76), Marchesini, and Ferrari ('96), Godlewski ('02), Mlodowska ('09) and numerous other writers, claim that the muscle fiber is of syncytial origin. The syncytium, most of these investigators believe, is formed by a secondary fusion of

independent epithelial myoblasts. Born ('73), Calberla ('76), Minot ('92), and Maurer ('06) give complete reviews of the literature, so a résumé of the papers of earlier investigators is unnecessary.

(3) The structure of the muscle of the adult œsophagus. Oppel ('97) gives a lengthy review of the literature on the structure of the muscle of the vertebrate œsophagus, to which the reader is referred. Since the structure of the adult muscle throws some light on the development, a short review of a few of the papers is given here. According to Oppel, all of the muscle of the œsophagus of amphibia, birds, and reptiles is smooth. In most fishes and mammals, a marked differentiation of smooth into cross-striated muscle has taken place. In some of the fishes both layers of muscle are striated throughout the length of the œsophagus; in other fishes only one layer, usually the thicker inner one is striated. Mammals also have varying amounts of striated muscle. In *ornithorhynchus*, all of the muscle in both layers is smooth, just as in amphibia, reptiles and birds. In a number of mammals (giraffe, elephant, all rodents, cattle and sheep) both layers of muscle are striated down to within one-fourth inch from the cardia. In some mammals striated fibers of the outer or longitudinal layer have been described as extending for a distance upon the cardia. Between these two extremes are all transitions.

Oppel states also that the primitive vertebrate probably possessed only smooth muscle in the œsophagus. The presence of striated muscle where it does occur, he thinks is due to a down-growth of the muscle of the pharyngeal constrictors upon the œsophagus. He thus derives the striated œsophageal muscle from the branchial muscle which is held by most investigators to arise from the lower head myotomes.

Coakley ('92) describes striated muscle fibers in the upper part of the human œsophagus as having a structure precisely like that of the skeletal muscle. In the lower œsophagus he found scattered striated fibers in both layers extending as far as the stomach. These lower fibers do not show as distinct cross-striations, as do the ordinary striated muscle fibers. He believes

that they are an intermediate form between smooth and cross-striated muscle. Though not so stated, he evidently thinks that the striated muscle here is formed directly from the smooth muscle.

Flint ('07) worked only on the grosser structure of the œsophagus of the pig embryo. The striated muscle is formed, he states, by differentiation of the mesenchyme. In the 13 mm. pig embryo the mesenchyme cells have begun to elongate. By the time the embryo is 7.5 cm. long, both layers of muscle are differentiated. The first evidence of cross-striation appears at 11 cm.

The writer ('07) in a description of the development of smooth muscle in the œsophagus of the pig, found the striated muscle developing somewhat earlier than described by Flint. There is an elongation of mesenchyme cells to form the circular layer beginning in the mid-œsophagus of the 5 mm. pig. In the 8 mm. embryo the elongation of cells extends the entire length of the œsophagus. From this differentiated mesenchyme, in the upper and mid-œsophagus, striated muscle develops; in the lower œsophagus, smooth muscle.

#### MATERIAL AND METHODS

The œsophagus of the dogfish (*Acanthias vulgaris*) and of the pig was the material used. Serial sections of dogfish embryos from 3 mm. to 60 mm. in length were studied. The embryos from 3 mm. to 10 mm. long were fixed in Zenker's fluid. All the longer ones were fixed in sublimate or in sublimate-acetic solutions. The pig embryos used ranged from 4 mm. to 60 mm. in length. They were all fixed in Zenker's fluid and were cut in serial sections. All were embedded in paraffin. The sections were stained in Delafield's hæmatoxylin, Heidenhain's iron-hæmatoxylin with a counter stain of Congo red, and in Mallory's anilin blue connective tissue stain.

#### OBSERVATIONS

This paper is restricted to the early development of striated muscle in the œsophagus. The later development, including the differentiation, growth, and multiplication of the fibers, is reserved for a separate paper.

For tracing the origin of the myoblasts, dogfish embryos were used. The later transformation of myoblasts into muscle fibers was studied chiefly in pig embryos.

#### (1) THE ORIGIN OF MYOBLASTS IN THE ŒSOPHAGUS

As already mentioned in the literature review, Oppel ('97) found the striated muscle of the œsophagus to be a downgrowth of pharyngeal muscles, hence arising indirectly from the myotomes of the branchial region. Flint ('07) and the writer ('07) state that it arises from the mesenchyme surrounding the endodermal tube.

In both dogfish and pig embryos, the œsophageal striated muscle arises directly from the mesenchyme. There is apparently no downgrowth from the pharyngeal region, at least in the dogfish. To be sure of this it was necessary to study early embryos and to trace the development of the mesenchyme cells in which the muscle arises. This was done in the dogfish material.

The earliest dogfish embryo studied by the writer was 3 mm. in length. At this stage there are very few mesenchyme cells formed. The mesoderm is represented by two layers, the somatic and splanchnic, which have extended dorsalward and form a number of myotomes. In places in the head region from the splanchnopleure at its junction with the myotome, a few irregular cells seem to be arising, which may represent the first mesenchymal cells. Minot ('92) stated that the mesenchyme arises solely from the mesothelium, the cells leaving the mesothelium, but remaining connected with it and with each other by protoplasmic processes. Thus from its origin the mesenchyme is a syncytium. He also found that the first mesenchyme of elasmobranchs arises from the splanchnic layer at the point where the myotome unites with the nephrotome.

Fig. 1. is a cross-section through the upper œsophagus of a 3 mm. dogfish embryo. There is nowhere in this embryo any distinct mesenchyme formed. At *a* in fig. 1. is a stellate cell which seems to have formed from the mesothelium, and is still connected with it by protoplasmic bridges. It may be one of



the earliest mesenchyme cells. The mesothelial cells at this time have numerous processes which extend to the surrounding organs. Fig. 4 is a high power drawing of area X in fig. 1, and shows these processes distinctly. They are most numerous dorsal and ventral to the coelom.

Fig. 2 is drawn from a section near the mid-oesophagus of the same 3 mm. embryo. The myotomes are shown connected with the coelomic mesothelium by a narrow strand of irregular cells. Under high power (fig. 5) these cells are found to be connected by wide anastomoses, and from them numerous processes extend to the ectoderm and the endoderm. They have much the appearance of mesenchyme cells, but sections of a later embryo at this region show that no true mesenchyme has yet been formed.

Fig. 3 is through the lower oesophagus of the same embryo as shown in figs. 1 and 2. At this point the two layers of the mesothelium are distinct and the coelomic cavity extends into the myotome. Fig. 6 is a high power drawing of area X in fig. 3. There are wide protoplasmic anastomoses between the two layers of mesothelial cells, and many finer processes extend to the basement membranes of the ectoderm and the endoderm. In the 3 mm. embryo the myotome is in contact with the epithelium of the oesophagus, but a study of later stages shows that before the mesenchyme, which later surrounds the oesophagus, is formed, the myotomes have grown some distance dorsalward and are well removed from the oesophagus. At a later stage, also, most of the protoplasmic processes of the mesothelial cells have been withdrawn, so it is probable that they do not represent the anastomoses of the later mesenchymal syncytium.

Since syncytium, as used in recent anatomical writings, has had various meanings, its definition as here employed is given. By syncytium is meant any tissue where there are well defined protoplasmic anastomoses between the cells. Where all the cells are so united, the tissue is described as a complete syncytium. Where some of the cells are independent and others are connected, the term partial syncytium is used. By cell, as employed in describing a syncytium, is meant merely the irregular stellate or spindle shaped mass of protoplasm which makes up a nodal

point of the protoplasmic network, together with the enclosed nucleus.

In the 4 mm. dogfish embryo the coelomic epithelium has grown far forward beyond the region of the oesophagus; and from it, around the brain and upper pharynx, considerable mesenchyme is forming by the outgrowth of stellate cells from the mesothelium. In the neighborhood of the oesophagus, the condition is almost precisely as in the 3 mm. dogfish, with the exception that the myotomes have grown farther dorsalward.

Fig. 7 is from the upper oesophagus of a 5.5 mm. dogfish embryo. Here the myotome is well formed and has grown well away from the endodermal tube. The mesothelium is distinctly epithelial and shows fewer cytoplasmic processes than were present in the 3 mm. embryo. Though no mesenchyme is yet present in this region, it is well formed as a complete syncytium filling in the spaces between the organs in the head region.

Fig. 8 is from the mid-oesophagus of the same embryo as shown in fig. 7. Here there are a few stellate cells between the splanchnic layer of the mesoderm and the endoderm, but the high power drawing (fig. 9) of area of X in fig. 8 shows that most of these loose cells are really endothelial cells. The blood vessels are growing in at this stage.

In the 10 mm. dogfish embryo there is the first distinct formation of mesenchyme between the splanchnopleure and the endoderm of the oesophagus. The splanchnic mesothelium is closely applied to the endoderm and also extends as a mesentery to become continuous with the somatic layer. The enlargement of the coelom brings about this condition as shown in fig. 10. At this stage the oesophagus is more definitely separated from the myotome and from the mesenchyme, *mc*. In the uppermost part of the oesophagus near the pharynx there is a very small amount of mesenchyme, continuous with the head mesenchyme. Longitudinal sections of this region show nothing that can be identified as cells of myotomic origin. The mesenchyme here comes from the splanchnopleure. At A in fig. 10, mid-oesophagus, is a spindle-shaped mesenchyme cell between the endoderm and the mesothelium. Fig. 11 is a high power drawing from a section of

the same embryo, slightly anterior to the one from which fig. 10 was taken. This section shows the origin of two mesenchyme cells from the mesothelium.

In fig. 12 is shown the mid-oesophagus of a 25 mm. dogfish. This is at the region where the lumen of the oesophagus is obliterated for a time. In sections both above and below the one pictured no lumen is present. Here quite a thick layer of mesenchyme has differentiated from the mesothelium. Longitudinal sections show that this mesenchyme forms *in situ*. There are no indications that its cells migrate from the myotomes, from which the oesophagus is now completely separated. Longitudinal sections show that in the upper oesophagus little or no mesenchyme has yet appeared.

The mesenchyme cells of the oesophagus form a complete syncytium. They are still connected by wide anastomoses with the mesothelium. From this time on, most of the new formation of the mesenchyme of the oesophagus in this region is by the mitosis of the cells already formed, and not by further differentiation from the mesothelium. In the mesenchymal syncytium of the mid-oesophagus the cells soon begin to differentiate into myoblasts, as shown in Fig. 13.

Pig embryos were not obtained young enough to trace the early formation of the myoblasts. When first studied in a 4 mm. pig embryo, the oesophagus and surrounding region show about the same structure as seen in the 25 mm. dogfish embryo (fig. 12). In the 4 to 7 mm. pig embryos the oesophagus is surrounded by a thick layer of undifferentiated mesenchyme. There is no indication of a migration of myoblasts from the myotomes into this tissue. As we have seen, it seems highly probable from the study of the early dogfish embryo that the myoblasts of the oesophagus arise from the mesenchyme derived from the splanchnopleure, not from the muscle plate, and that there is no downgrowth of the pharyngeal muscle to form oesophageal muscle. The same condition is probably present in the pig embryo.

## (2) THE TRANSFORMATION OF THE MESENCHYME INTO CROSS-STRIATED MUSCLE

### (a) *Early Development*

In the dogfish mid-œsophagus, until the embryo reaches a length of 26 mm., and in the pig embryo until it reaches 5 mm. the tissue outside of the endodermal tube consists of a loose mesenchymal syncytium, the origin of which has just been described. The nuclei of the syncytium are round or oval with distinct nuclear wall and chromatin reticulum. From one to three true nucleoli are present in each nucleus, but they are frequently obscured by the chromatin. The protoplasm shows a reticular structure, the strands of which are made up of fine granules (fig. 12 from a 30 mm. dogfish embryo, and fig. 15 from a 7 mm. pig embryo).

In this mesenchymal syncytium throughout the entire length of the dogfish œsophagus, and in the upper two-thirds of the pig œsophagus, striated muscle develops. In the lower third of the pig œsophagus smooth muscle develops. The first stage in muscle formation is a general condensation of the mesenchyme at a short distance from the endodermal tube. This begins in the 4 mm. pig embryo, and in the 25 mm. dogfish embryo. The condensation is brought about by a rapid mitotic division of the nuclei, with a corresponding increase in the amount of syncytial cytoplasm. The condensation begins in the mid-œsophagus and extends rapidly both up and down the tube.

The early formation of striated muscle in the œsophagus of both pig and dogfish up to the time the cross striations appear in the fibrillæ, is precisely like the development of the smooth muscle of the lower œsophagus of the pig. The two tissues arise from a continuous sheet of mesenchyme. The description given in the writer's paper on the histogenesis of the smooth muscle in the œsophagus of the pig (McGill '07) will therefore apply equally well for the early development of the striated muscle in this region. A comparison of figs. 1 to 21 of the earlier paper with figs. 1 to 17 of the present paper will show this striking similarity. It will be necessary merely to compare the formation

of striated muscle with that of smooth muscle already described and to refer the reader to the writer's previous paper for details of the early myogenesis.

Precisely as in smooth muscle histogenesis, when the formation of striated muscle of the œsophagus begins in the condensed mesenchyme, it is first of all indicated by an elongation of some of the mesenchymal nuclei. For the circular layer of muscle, this begins in the 5 mm. pig embryo. The protoplasm around each nucleus increases in amount and likewise elongates (figs. 16 to 18). Elongation for the formation of the longitudinal layer does not begin in the pig until the embryo reaches a length of 20 mm.

Here, too, as in smooth muscle formation, the statement that muscle arises from undifferentiated mesenchyme is true only for the first few myoblasts formed. In the 15 mm. pig throughout the mesenchyme collagenous fibers appear, as shown by Mallory's stain. Most of the striated muscle arises from this embryonal connective tissue just as does the smooth muscle. Some of the embryonal connective tissue cells in the areas of muscle formation remain undifferentiated and form the interstitial connective tissue both of smooth and striated muscle.

#### *(b) Increase in the Number of Myoblasts*

The increase in the number of myoblasts of striated œsophageal muscle takes place in just the same way as does that of smooth muscle. That is, either by a continuation of the transformation of the embryonal connective tissue cells into myoblasts, or by the mitotic division of myoblasts already formed. Seldom do mitoses occur in the myoblasts after many myofibrillæ have appeared.

#### *(c) The Formation of Myofibrillæ*

Immediately following the elongation of the mesenchymal nuclei, or later, of the embryonal connective tissue nuclei, the myofibrillæ arise in the cytoplasm. The myofibrillæ, both in the dogfish and in the pig, develop as homogeneous structures without cross-striations. They look exactly like the early

fibrillæ of smooth muscle. This agrees with the observations of Bardeen ('00), Godlewski ('02) and Eycleshymer ('04).

Two varieties of homogeneous myofibrillæ form, the coarse and the fine. The coarse myofibrillæ arise in the granular cytoplasmic reticulum.. Many of the coarse protoplasmic granules which are present in large numbers at the time the coarse myofibrillæ first appear, seem to be of nuclear origin. As the mesenchymal nuclei in the area of muscle formation multiply by mitosis some of the chromatin appears to be left outside in the cytoplasm (figs. 20, 22, 23). These coarse granules become arranged in clumps to form spindle shaped masses. These spindles are usually close to the nuclei (figs. 18 and 21). The granules in the spindles soon fuse to form homogeneous structures. Neighboring spindles unite to form long, varicose fibrillæ (fig. 21).

Mlodowska ('08) has described a similar process in skeletal muscle. These coarse, varicose fibrillæ extend long distances through the protoplasmic syncytium. In the 15 mm. pig embryo some of them extend over half the circumference of the œsophagus. In time the fibrillæ become more nearly uniform in caliber (fig. 17). This type of formation of coarse fibrillæ is found only in the early embryo. All subsequent myofibrillæ arise as fine fibrillæ which later thicken to form uniform coarse fibrillæ. The development of coarse myofibrillæ begins in the 9 mm. pig embryo (fig. 16) and in the 30 mm. dogfish embryo (fig. 13).

The formation of fine myofibrillæ begins in the 25 to 30 mm. pig embryo. Their development is practically like that of the fine fibrillæ of smooth muscle. In striated muscle, however, all of them later form coarse fibrillæ.

In fig. 25 an interesting stage is seen. Here the first formed coarse fibrillæ have become cross-striated. Among them are other coarse fibrillæ not yet striated, and also numerous fine myofibrillæ just arising. On the periphery of the muscle layer is embryonal connective tissue differentiating into muscle. The myofibrillæ here arising are fine in the beginning, not coarse as were the first myofibrillæ. These fine myofibrillæ gradually thicken and finally also become cross-striated. The first-formed coarse and fine myofibrillæ correspond very closely to the coarse

and fine fibrillæ found in developing smooth muscle. In fact, in fig. 17 from a section through the upper œsophagus of a 15 mm. pig embryo, the developing cross-striated muscle has precisely the same appearance as has the developing smooth muscle of the lower œsophagus of the same embryo.

The first evidence of cross-striation in the pig was seen in the 13 mm. embryo in the muscle of the circular layer of the mid-œsophagus. Few of the homogeneous fibrillæ however become cross striated before the embryo reaches a length of 25 mm. Cross-striations were seen in the longitudinal muscle layer of the 27 mm. pig embryo. In the dogfish, cross striations appear in the muscle of the œsophagus in embryos between 50 mm. and 60 mm. in length.

In the development of the striated muscle of the œsophagus, just as in the smooth muscle, the myofibrillæ arise everywhere in a syncytium. The syncytium in striated muscle persists until a late stage, when it is partially broken up to form the muscle fibers. This takes place when the sarcolemma differentiates from the interstitial connective tissue. Even after the muscle fibers are formed, the syncytium is in part retained, for each muscle fiber is derived from several cells of the original syncytium.

The nuclei seem to take an active part in the formation of myofibrillæ. In their division, as already mentioned, they seem to leave at times much chromatin behind in the cytoplasm, and this chromatic material helps to form the first myofibrillæ. Then at the time the fibrillæ are forming most rapidly the muscle nuclei are filled with deeply staining chromatin (figs. 17 and 22). In all of these early stages the muscle nuclei stain much more deeply than do the connective tissue nuclei, unless the latter be in mitosis. The fact that in their development the myofibrillæ begin to arise near the nuclei, and that the spindle-like enlargements of the varicose fibrillæ are usually near the nuclei, is also evidence that the nuclei may take part in fibrillar formation. Now and then in early myogenesis some of the muscle nuclei seem to break down completely and liberate their chromatin into the cytoplasm. This chromatin also may possibly take part in fibrillar formation.

*(d) The Interstitial Connective Tissue*

The early formation of the interstitial connective tissue is very similar in the striated muscle of the œsophagus to that already described for the smooth muscle, so the details of development are not given here. The connective tissue arises *in situ*. In skeletal muscle it does not grow in from the outside as has been described by most recent workers, Bardeen ('00), Godlewski ('02), Eycleshymer ('04), Mlodowska ('08), along with many early investigators. There is also no indication in the histogenesis of œsophageal striated muscle of a degeneration of the forming muscle tissue to allow the ingrowth of the connective tissue, as has been found on skeletal muscle by Mayer ('86), Bardeen ('00), Godlewski ('02), Eycleshymer ('04), and Mlodowska ('08).

In development, protoplasmic anastomoses between the muscle cells and the connective tissue cells, are everywhere demonstrable (figs. 13, 14, 16, 17, 18, 22 and 24). In this protoplasmic syncytium, myofibrillæ and connective tissue fibrillæ develop side by side. Later, the collagenous fibrillæ are crowded out of the muscle protoplasm by the growth of myofibrillæ. Numerous figures from material stained in Mallory's anilin blue connective tissue stain, showing the differentiation of the collagenous and myo-fibrillæ side by side, are given in the writer's previous paper.

*(e) The Relation of Striated to Smooth Muscle*

The origin of the œsophageal muscle as traced in the pig and the dogfish embryo seems to confirm Oppel's statement that the smooth muscle is the primitive muscle of the œsophagus. Oppel arrived at his conclusion from the standpoint of comparative anatomy and phylogeny. In the pig and in the dogfish œsophagus, both tissues as we have seen, arise side by side from the common mesenchymal syncytium. Until the cross-striations appear in the fibrillæ of the striated muscle, both developing tissues look precisely alike. Smooth muscles may retain nearly this primitive structure in the adult. In the lower œsophagus of the pig the adult muscle retains its syncytial structure and has in places practically the same appearance as the embryonal



syncytium in the formation of cross-striated muscle shown in fig. 17. In most places however, more myofibrillæ develop in the smooth muscle syncytium, and there is later in the myofibrillæ of smooth muscle a tendency to be grouped to form individual spindle-shaped muscle fibers or cells. As far as I have found, all the transitions from smooth to cross-striated muscle in vertebrates occur only in the embryo. There is a possibility, however, that even in the adult mammal, intermediate forms between smooth and cross-striated muscle may occur, as Coakley ('92), described in the human œsophagus. At any rate, in development the two tissues are very closely related.

#### SUMMARY

1. The tissue destined to form the striated muscle of the œsophagus (dogfish) arises from the splanchnic layer of the mesothelium in the region where this epithelium is in contact with the œsophageal endoderm. Apparently there is no connection at any stage of development between this muscle-forming tissue, which is typical mesenchyme, and the cells of the myotome.

2. The mesenchyme in which striated muscle of the œsophagus forms, in both pig and dogfish, is a complete syncytium.

3. In the 4 mm. pig embryo and in the 25 mm. dogfish embryo there is a condensation of the mesenchyme around the endoderm of the œsophagus. In this condensed mesenchyme the muscle arises.

4. The next step in muscle differentiation is an elongation of some of these mesenchymal nuclei accompanied by an increase in the amount of the surrounding cytoplasm. This begins in the œsophagus of the 5 mm. pig embryo and of the 30 mm. dogfish embryo.

5. After the first formation of muscle, the tissue increases in amount in two ways: (1) by addition of new myoblasts from the mesenchyme without, or by differentiation of interstitial embryonal connective tissue cells into myoblasts; and (2) by mitotic division of the myoblasts already formed.

6. As the nuclei elongate in the muscle-forming tissue the

myofibrillæ arise in the protoplasmic syncytium. The fibrillæ form as homogeneous structures, which later become cross-striated. In first formation they are of two types, coarse and fine.

7. The coarse myofibrillæ form first and develop by a massing of protoplasmic granules into irregular spindle-shaped structures. The spindles form near the nuclei. Soon the spindles unite end to end to form varicose fibrillæ. The granules fuse and the fibrillæ become homogeneous and later of uniform caliber. Shortly after this the cross-striations appear. The coarse myofibrillæ arise in the 9 mm. pig embryo and in the 30 mm. dogfish embryo.

8. In the older embryos all of the fibrillæ form as fine myofibrillæ. These increase in size and later may form coarse myofibrillæ.

9. Cross-striations were first distinguished in the œsophagus of the 13 mm. pig embryo and of the 50 mm. dogfish embryo. In the pig, however, only a few fibrillæ become striated before the embryo reaches a length of 30 mm.

10. The nuclei appear to play an active part in the formation of myofibrillæ.

11. The interstitial connective tissue of the œsophageal striated muscle is formed *in situ* from embryonal connective tissue cells, which remain undifferentiated among the muscle cells. There is thus no necessity for ingrowth of connective tissue such as is described in the histogenesis of skeletal muscle.

12. There is at no stage in the development of the striated muscle of the œsophagus a degeneration of muscle cells such as some investigators have found in the histogenesis of skeletal muscle.

13. The muscle tissue remains a complete syncytium until a comparatively late stage, when the interstitial connective tissue grows rapidly in connection with the formation of the definite cross-striated muscle fibers.

14. The smooth and the cross-striated muscle of the œsophagus arise from a common mesenchymal syncytium. In the early stages, up to the time when the cross-striations form, both tissues appear identical in structure. The striated muscle of the œsophagus seems to be only a further differentiation of smooth muscle.

No transition forms between the two tissues however were found in the adult œsophagus.

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## EXPLANATION OF FIGURES

## ABBREVIATIONS

ao	aorta
b ec	basement membrane of ectoderm
c	cœlom
c mf	coarse myofibrilla
c nu	connective tissue nucleus
cap	capillary
ec	ectoderm
ect	embryonal connective tissue
en	endoderm
et	endothelium
f mf	fine myofibrilla
g mf	granular myofibrilla
h	heart
m	myotome
mc	mesenchyme
mit	mitotic nucleus
m nu	muscle nucleus
ml	mesothelium
mu	muscle
n	notochord
nc	nerve cord
p cr	cytoplasmic chromatin
p s	protoplasmic syncytium
s mf	cross-striated myofibrilla
sp mf	spindle of developing myofibrilla.

FIG. 1. Cross-section through the body of a 3 mm. dogfish embryo at level of upper œsophagus. There is no mesenchyme formed. The mesothelial cells, *mt*, show fine protoplasmic processes, *a* is a loose cell which appears to be a mesenchyme cell just arising from the mesothelium. Zenker's fluid, iron-hæmatoxylin. B. and L. oc. 10, obj. 16 mm.

FIG. 2. Cross-section through same embryo as in fig. 1, but taken at the level of mid-œsophagus. The myotome, *m*, is formed. Between the myotome and the cœlomic epithelium, *mt*, is a strand of loose cells resembling mesenchyme. Zenker's fluid iron-hæmatoxylin. B. and L. oc. 10, obj. 16 mm.

FIG. 3. A section through the lower œsophagus of the same embryo. The connection of the cavity of the myotome with the cœlom is shown. Zenker's fluid, iron-hæmatoxylin. B. and L. oc. 10, obj. 16 mm.

FIG. 4. A high power drawing of area *X* in fig. 1 to show the protoplasmic network extending from the mesothelial cells to the surrounding organs. B. and L. oc. 10, Zeiss 2. mm. 1.30 apochrom. obj.

FIG. 5. A high power drawing of region *X* in fig. 2. The mesothelial cells are more or less united into a syncytium. B. and L. oc. 10, Zeiss 2. mm. 1.30 apochrom. obj.

FIG. 6. A high power drawing of region *X* in fig. 3. The mesothelial cells are more or less fused leaving only a partially developed cœlom. The mesothelium forms two layers, splanchnic and somatic. Protoplasmic processes are numerous. B. and L. oc. 10, Zeiss 2 mm. 1.30 apochrom. obj.

FIG. 13. Section through the mid-œsophageal mesenchyme of a 30 mm. dogfish embryo; *b en*, basement membrane of endoderm. Just outside this basement membrane is a thick layer of mesenchymal syncytium made up of stellate cells joined by wide protoplasmic anastomoses. At *mu*, some of the mesenchymal nuclei are elongating to form muscle nuclei; *mf*, developing myofibrilla. Sublimated acetic, iron-hæmatoxylin. B. and L. oc. 10, Zeiss 2 mm. 1.30 apochrom. obj.

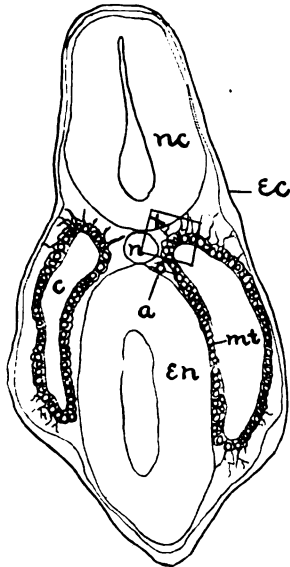


Fig. 1

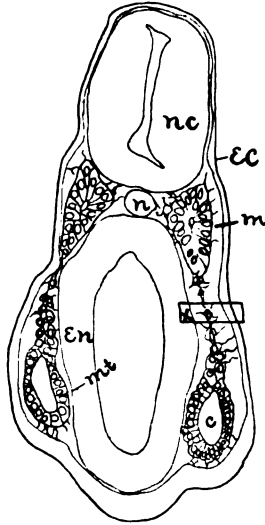


Fig. 2

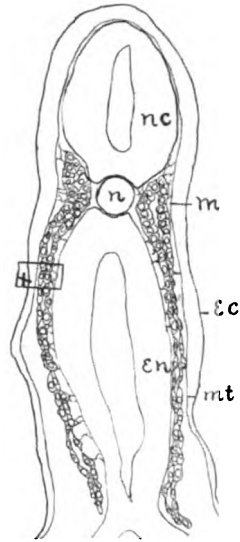


Fig. 3

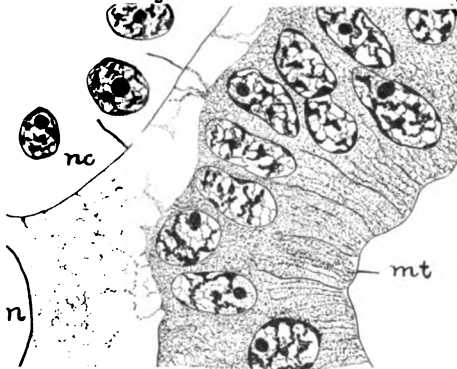


Fig. 4

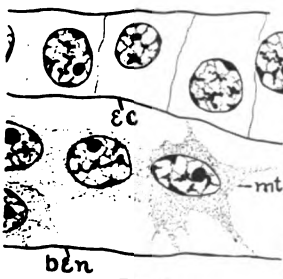
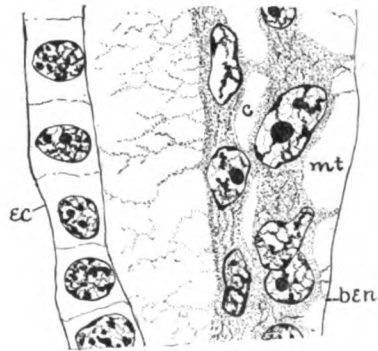


Fig. 5

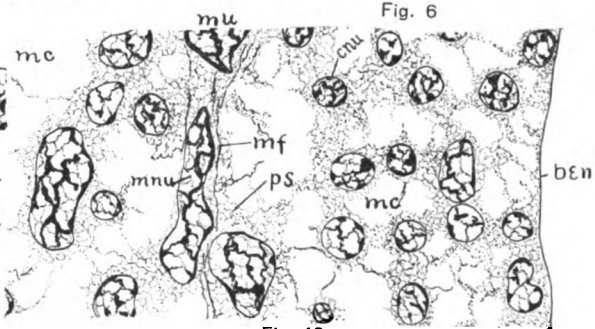


Fig. 13

FIG. 7. Cross-section of a dogfish embryo 5.5 mm. in length, at the upper œsophagus. The myotome has grown dorsal to the endodermal tube, *en*. The rest of the mesoderm is represented by the double layer of mesothelium. The coelom is only a narrow slit between the two layers of mesothelium. Zenker's fluid, iron-hæmatoxylin. B. and L. oc. 10, obj. 16 mm.

FIG. 8. Through mid-œsophagus of a 5.5 mm. dogfish embryo. The mesoderm is similar to that of the upper œsophagus shown in fig. 7. The blood vessels are growing in, so there is considerable endothelium present, *et*. B. and L. oc. 10, obj. 16 mm.

FIG. 9. A high power drawing of area *X* in fig. 8. The drawing extends from the basement membrane of the endoderm, *b en*, to that of the ectoderm, *b ec*. The middle germ layer here is represented by the two layers of mesothelium and the endothelium of the blood vessels. No mesenchyme has formed in the region. B. and L. oc. 10, Zeiss 2 mm. 1.30 apochrom. obj.

FIG. 10. A section through mid-œsophagus of a 10 mm. dogfish embryo. The endodermal tube is suspended by a double layer of mesothelium. *A* is a mesenchyme cell differentiating from the splanchnopleure. Considerable mesenchyme has formed between the somatic mesothelium and the body-wall and also around the myotome. The muscle plate is well removed from the œsophagus. *h*, heart; *c*, coelomic cavity; *ao*, aorta. Zenker's fluid, iron-hæmatoxylin. B. and L. oc. 10, obj. 16 mm.

FIG. 11. Section through the splanchnic mesothelium and developing mesenchyme of a 10 mm. dogfish embryo near the region shown in fig. 10. At *mc* are mesenchyme cells which are being formed from the mesothelium. B. and L. oc. 10, Zeiss 2 mm. 1.30 apochrom. obj.

FIG. 12. Cross-section through the mid-œsophagus of a 25 mm. dogfish embryo. The œsophagus is suspended in the coelomic cavity well separated from the other organs. Here an amount of mesenchyme has formed between the mesothelium and the endoderm. The mesenchyme forms a syncytium. The cells are stellate with round or oval nuclei. Sublimate-acetic, iron-hæmatoxylin. B. and L. oc. 5, obj. 6 mm.

FIG. 14. Section through the circular muscle layer of the mid-œsophagus of a 60 mm. dogfish. Shows various stages in the differentiation of the myofibrillæ. Cytoplasm forms a complete syncytium. At the margins, the embryonal connective tissue is differentiating into muscle. Sublimate-acetic, iron-hæmatoxylin. B. and L. oc. 10, Zeiss 2 mm. 1.30 apochrom. obj.

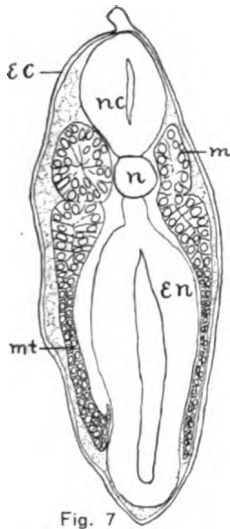


Fig. 7

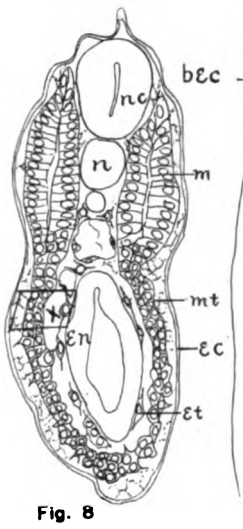


Fig. 8

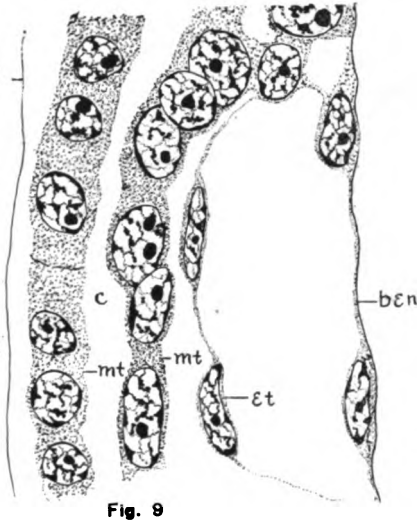


Fig. 9

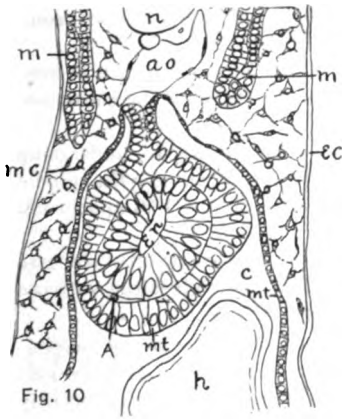


Fig. 10

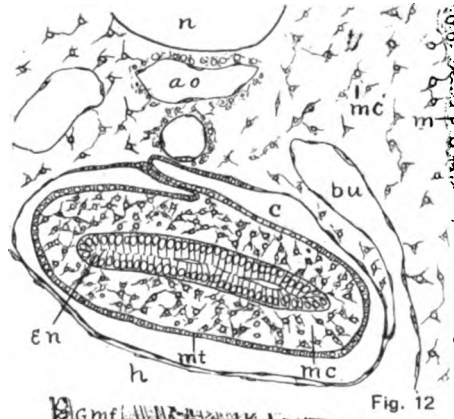


Fig. 12

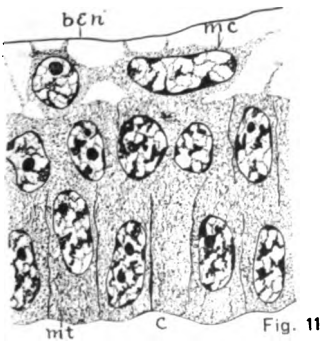


Fig. 11

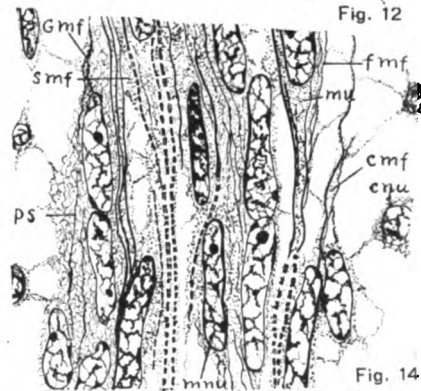


Fig. 14



FIG. 15. Cross-section through the œsophagus and surrounding tissue of a 7 mm. pig. embryo. Note the condensed mesenchymal syncytium with a few of the nuclei concentrically arranged. *b v*, blood vessel; *tr*, trachea; *oes*, œsophagus. Zenker's fluid, iron-hæmatoxylin. Zeiss oc. 4, obj. D.

FIG. 16. Section through a portion of the œsophagus of a 10 mm. pig embryo. This shows the condensation of the cytoplasm and elongation of mesenchymal nuclei to form the circular muscle coat. *g mf*, coarse cytoplasmic granules arranging in rows, the first indication of the coarse myofibrillæ. *mit*, shows mitosis in the muscle-forming tissue. Zenker's fluid, iron-hæmatoxylin. Zeiss comp. oc. 8, 2 mm. 1.30 apochrom. obj.

FIG. 18. Small portion of the muscle syncytium from the œsophagus of a 13 mm. pig embryo showing the origin of the coarse myofibrillæ from the granular reticulum. *gmf*, is a mass of coarse chromatic (?) granules; at *sp mf*, the granules have almost completely fused to form a homogeneous, spindle-shaped mass. *a* shows a granular strand connecting this spindle with another smaller one. *b* is a myofibrilla arising in part in the protoplasm of an interstitial connective tissue cell. Zenker's fluid, iron-hæmatoxylin. Zeiss comp. oc. 12, 2 mm. 1.30 apochrom. obj.

FIG. 19. A nucleus from muscle-forming tissue in the œsophagus of a 13 mm. pig, in prophase of mitosis. It shows the large deeply staining chromosomes. Zeiss comp. oc. 8, 2 mm. 1.30 apochrom. obj.

FIG. 20. A nucleus from the same section as that shown in fig. 19, undergoing mitosis. A large amount of chromatin (*pcr*) apparently has not entered the spindle but remains outside in the cytoplasm. Zeiss comp. oc. 8, 2 mm. 1.30 apochrom. obj.

FIG. 21. From the same region as fig. 20. This shows the formation of a coarse myofibrilla by the union of spindles near several nuclei. This gives the varicose appearance. Zeiss comp. oc. 12, 2 mm. 1.30 apochrom. obj.

FIG. 23. Two mitotic nuclei from the region of muscle formation in the œsophagus of a 13 mm. pig embryo. Nucleus *a* is in prophase and shows a large amount of chromatin. Nucleus *b* is in telophase. Many chromatic granules are apparently left in the cytoplasm. Zenker's fluid, iron-hæmatoxylin. Zeiss comp. oc. 8, 2 mm. 1.30 apochrom. obj.

FIG. 24. Muscle tissue from the œsophagus of a 13 mm. pig embryo. The coarse myofibrilla at *s mf* is beginning to show cross striations. Zenker's fluid, iron-hæmatoxylin. Zeiss comp. oc. 8, 2 mm. 1.30 apochrom. obj.

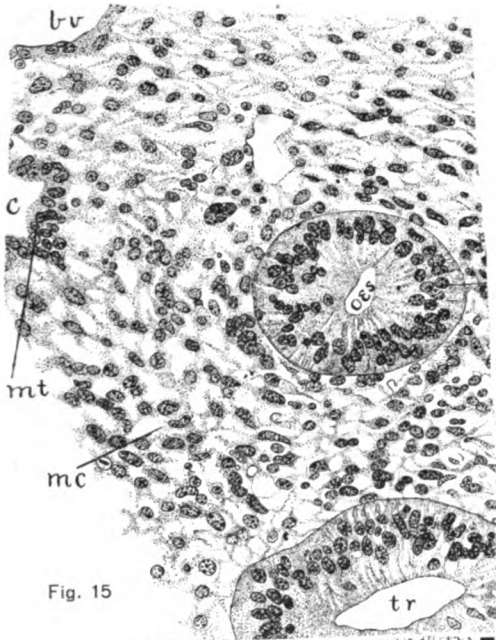


Fig. 15

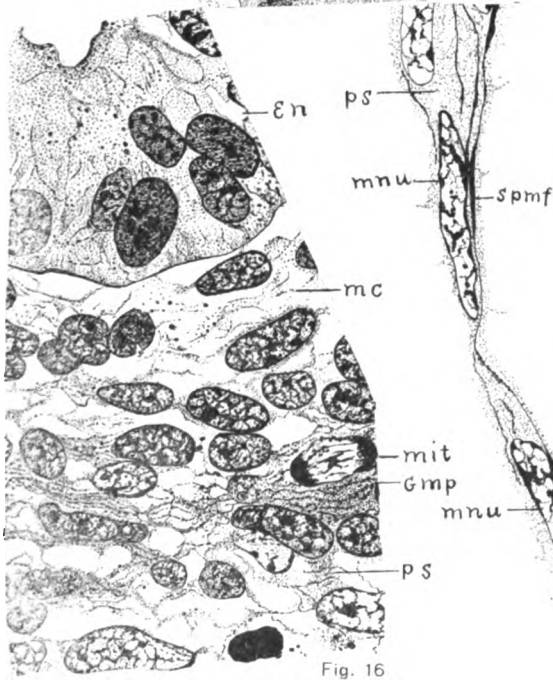


Fig. 16

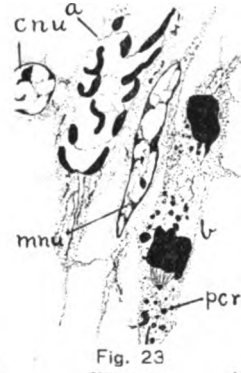


Fig. 23

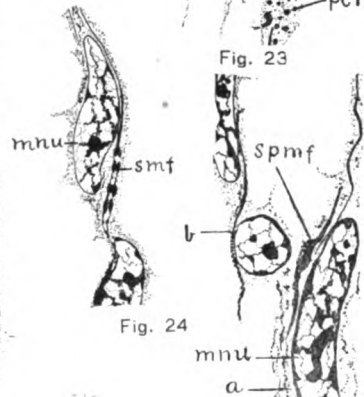


Fig. 24

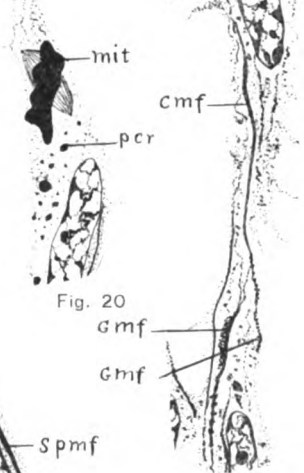


Fig. 20

Fig. 18



Fig. 19

FIG. 17. Through a portion of the wall of the œsophagus of a 15 mm. pig embryo near bifurcation of trachea to show area of cross-striated muscle formation. Cytoplasmic syncytium everywhere present. The myofibrillæ show in various stages of development. *p cr*, chromatic (?) granules free in the protoplasm. The coarse myofibrilla, *c mf*, ran over one-half way around the œsophagus. Many of the fine fibrils visible in the mesenchyme are collagenous fibrils which can be differentiated with Mallory's anilin blue connective tissue stain. Zenker's fluid, iron-hæmatoxylin. Zeiss comp. oc. 12, 2 mm. 1.30 apochrom. obj.

FIG. 22. The muscle syncytium from the mid-œsophagus of a 15 mm. pig embryo. The large amount of chromatin (?) in the protoplasmic syncytium is noticeable, *pcr*. The muscle nuclei stain very intensely. Zenker's fluid, iron-hæmatoxylin. B. and L. oc. 10, Zeiss 2 mm. 1.30 apochrom. obj.

FIG. 25. A section through the inner part of the circular muscle layer of a 27 mm. pig œsophagus. This section shows a number of stages in the formation of myofibrillæ. The oldest fibrillæ are cross-striated, *s mf*. *c mf* is a coarse myofibrilla only in part striated. *f mf* are fine fibrillæ just arising. In the adjacent embryonal connective tissue numerous cells are elongating to form muscle. With Mallory's anilin blue connective tissue stain, these cells are found to contain both myofibrillæ and collagenous fibrils. *Cap* is a capillary. Zeiss comp. oc. 8, 2 mm. 1.30 apochrom. obj.

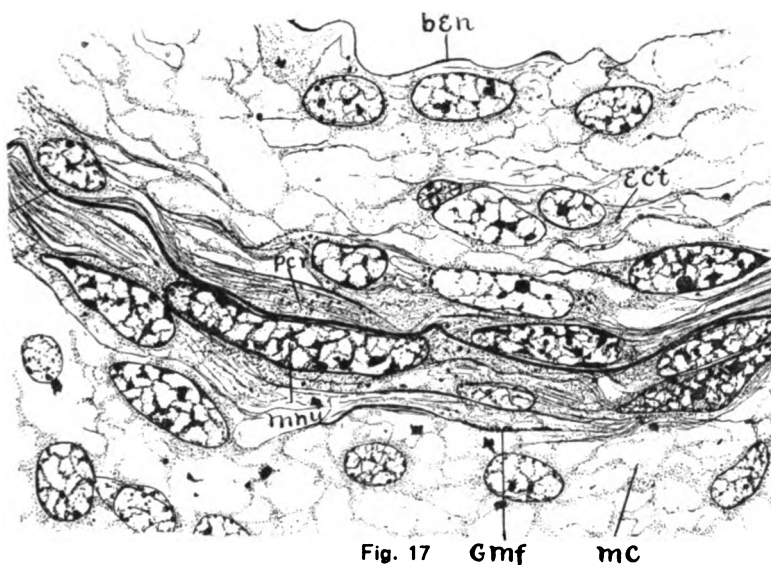


Fig. 17

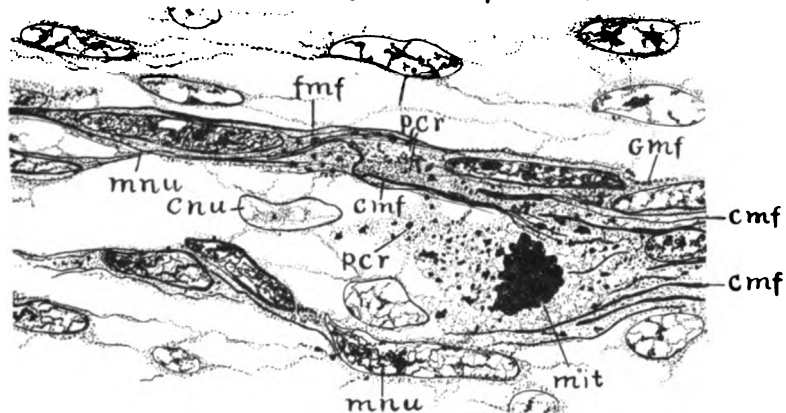


Fig. 22

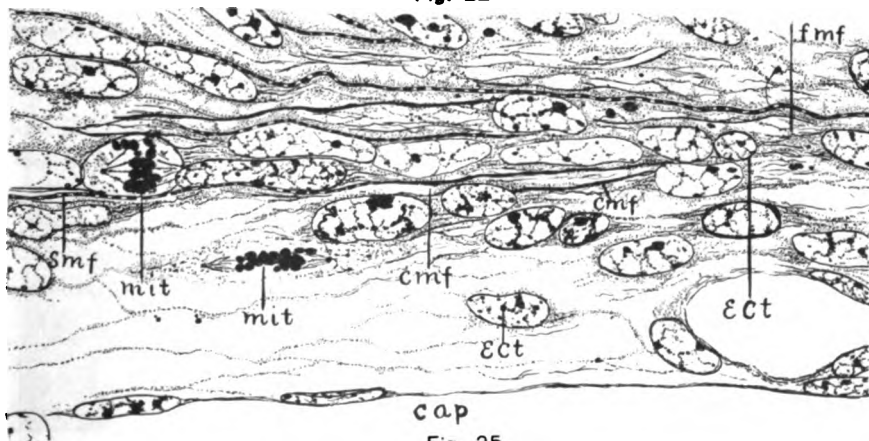


Fig. 25



## BOOK REVIEW

**A TEXT-BOOK OF ANATOMY.** Edited by D. J. Cunningham, F.R.S.  
Third Edition, 1909. New York: William Wood and Company.

The revision of the third edition of Cunningham's Text-Book of Anatomy was the last labor of its distinguished editor. The style and plan of the third edition remain the same as in previous editions. The sections on Osteology and Myology have been largely rewritten and the descriptive matter altered to conform to the BNA terminology and much has been gained thereby in clearness of style and conciseness of expression. It is to be hoped that the remaining sections of the book will undergo revision in the near future and the use of the BNA nomenclature consistently followed.

The number of pages in the section on Osteology remain the same as in the second edition, with thirty-four additional illustrations. In the description of the bones of the extremities new drawings are introduced, delineating in color the origin and insertion of the muscles. The bones entering into the construction of the skull as a whole, and in different planes of section viewed from various aspects, have been differentiated in this manner. Color likewise has been used to distinguish the articular surfaces of the bones of the hand and foot. There are several new radiographs of the foetal hand and foot.

Appended to the section on Osteology are six short but comprehensive accounts of: (A) Architecture of the bones of the skeleton; (B) Variations of the skeleton; (C) Serial homology of the vertebræ; (D) Measurements and indices employed in physical anthropology; (E) Development of the chondrocranium and morphology of the skull; (F) Morphology of the limbs.

The number of pages in the section on Myology are slightly increased and the new drawings are excellently executed. Many of these in their handling and representation show the influence of Spalteholz's Atlas. Of the drawings of the sole of the foot, three are taken from the left side and one from the right. Further, figures 294 and 301 are placed on the page with the digital extremities away from the observer, while figures 303 and 304 have a reversed position. The same discrepancy exists in the representation of the palmar aspect of the hand. The figures do not show clearly the manner of insertion of the flexor tendons and their disposition after entering the flexor sheaths.

A "Glossary of Anatomical Terminology" is prefaced to the introduction, giving a short historical account of the Basle Nomina Anatomica.

HENRY W. STILES.

## PRELIMINARY PROGRAMME

OF THE II. INTERNATIONAL ANATOMICAL CONGRESS,  
BRUSSELS, AUGUST 7-11, 1910.

SUNDAY, AUGUST 7

4:30 p.m. Session of the committee, consisting of the presidents and secretaries of the five united societies, and also the president and secretary of the local committee, to be held in the Anatomical Laboratory, Park Leopold: entrances rue Belliard and rue du Walbeek.

8:30 p.m. Welcome in a place to be announced.

MONDAY, 8th; THURSDAY, 11th.

Mornings, from 9 to 1. Sessions.

Afternoons, from 3 to 6. Demonstrations.

The scientific sessions will take place in the auditorium of the Physical Institute of the University, 14 rue des Sols.

The demonstrations will be held in the Physical Institute, Park Leopold.

The local committee consists of Messrs. Rommelaere, president of the Administrative Council of the University; Paul Ererra, Rector of the University, and Raoul Warocque, the founder of the Anatomical Institute, as honorary president, Professor Brachet as president, and Professor Joris as vice president.

Committee on Lodgings: Dr. Brunin, Chef des travaux (Anatomie).

Information concerning anatomy, comparative anatomy, and embryology may be obtained from Professor Brachet, rue Sneeessens 18; for histology, Professor Joris, rue de President 73.

A banquet is proposed for Wednesday, August 10.

## SYMPOSIUM ON COMPARATIVE NEUROLOGY<sup>1</sup>

### 1. THE PHYLOGENETIC ORIGIN OF THE NERVOUS SYSTEM

G. H. PARKER

*Harvard University*

The highly differentiated nervous system, such as is found in the vertebrates and other higher metazoans, is described as composed for the most part of many inter-related reflex arcs. Each of these arcs involves at least three parts: a sense organ or receptor which receives the external stimulus and originates the nervous impulse; a central nervous organ or adjustor in which the impulse may be variously modified and directed; and a muscle, gland, or other effector by which the animal responds to the external stimulus. Nerve fibers connect, of course, the receptor with the central apparatus and the latter with the effector. At least two classes of neurones are concerned in this mechanism, one afferent or sensory and the other efferent and usually motor. The sensory neurone is modified at its peripheral end to form the receptor and its nerve fiber extends as a rule to the central organ in which it rami-fies; its cell-body may occupy a peripheral position even forming an essential part of a sense organ as in many invertebrates and in the olfactory organ of vertebrates, or it may be nearly central in location as in the spinal ganglia of the vertebrates. The motor neurone has its cell-body within the central organ and its fiber as an efferent fiber extends to a muscle where it terminates. Besides these two classes of neurones, afferent and efferent, the central organs usually contain a vast congregation of correlation neurones which in one way or another intervene between those already mentioned.

<sup>1</sup>Presented at the twenty-fifth session of the American Association of Anatomists, Boston, December, 1909.



Such a nervous system as the one just described is found in the vertebrates, mollusks, anthropods, worms, and other higher metazoans, but is only feebly represented, if in fact it can be said to be represented at all, in the echinoderms, ctenophores, and coelenterates. The part that is least developed in these lower animals is the central organ, and, though this part cannot always be said to be absolutely unrepresented, it is so deficient, especially in the coelenterates, as to have led to the designation of their nervous apparatus as diffuse rather than centralized. The so-called diffuse nervous system of these animals is the simplest nervous system with which we are acquainted, for, notwithstanding repeated efforts, no true nervous structure has ever been demonstrated in those metazoans which, like the sponges, are more primitive than the coelenterates. If, therefore, the beginnings of the nervous system are to be sought for, attention must be directed to the coelenterates.

The coelenterate body is composed chiefly of two specialized epithelial layers, ectoderm and entoderm, each of which contains both nervous and muscular elements. The nervous elements are epithelial sense-cells whose receptive ends are at the periphery of the layer in which they are contained and whose nervous ends form a system of extremely fine interlacing branches many of which are probably directly connected with the deep-seated muscle-cells. The fine branches from many neighboring sense-cells establish what is probably a true nervous net by which transmission is accomplished not only to the subjacent muscle-cells but to those some distance away. Here and there this net contains conspicuous, multipolar cells which contribute fibrils to it and which for this reason are believed to be nervous. It is with the origin of this relatively simple neuromuscular mechanism that we are concerned.

In 1872 Kleinenberg announced the discovery in the fresh-water hydra of what he designated as the neuromuscular cell. The peripheral end of this cell was situated on the exposed surface of the epithelium of which it was a part and was believed to act as a nervous receptor; the deep end was drawn out into a muscular process and served as an effector to which transmission was supposed to be accomplished through the body of the cell. Each such cell was

regarded as a complete and independent neuromuscular mechanism, and the movements of an animal provided with these cells were believed to depend upon the simultaneous stimulation of many such elements. It was Kleinenberg's opinion that these neuromuscular cells divided and thus gave rise to the nerve-cells and muscle-cells of the higher animals. In fact he declared that the nervous and muscular systems of these animals were thus to be traced back to the single type of cell, the neuromuscular cell, which morphologically and physiologically represented the beginnings of both.

Some years later, in 1878, the Hertwigs published an account of the minute structure of the coelenterate nervous system and showed that Kleinenberg's so-called neuromuscular cells were probably merely muscle-cells in process of differentiation. They consequently proposed for these cells the more appropriate name of epithelio-muscle cell. They also claimed that in the evolution of the neuromuscular mechanism in coelenterates the three types of cells that they had identified, the sense-cells, ganglion-cells, and muscle-cells, were simultaneously differentiated from ordinary epithelial cells. Thus these three elements, though regarded as derived from a common layer, were, according to the Hertwigs, not the descendents of any single type of cell such as the neuromuscular cell.

Although Kleinenberg's theory and the theory of the Hertwigs differ in certain important details, they agree in declaring for the simultaneous and interrelated evolution of nerve and muscle. As contrasted with this view is the hypothesis that was first advocated by Claus and later by Chun that the two types of tissue arose independently and became secondarily united. In 1880 Chun called attention to the fact that in vertebrates the motor nerve-fibers grow out of the medullary tube and become connected with the muscles secondarily and he regarded this as evidence that nerve and muscle had arisen in phylogeny independently and had become secondarily united. But the majority of investigators have sided with the opinion expressed by Samassa (1892) that a nervous system purely receptive in function and without effectors of any kind is practically inconceivable. Hence the hypothesis of Claus and Chun has been generally regarded as untenable.

The current opinion among investigators as to the evolution of the nervous system of primitive metazoans remains essentially that of the Hertwigs, namely that nerve and muscle have been differentiated simultaneously and in close physiological interrelation but from cells which were separate members of an epithelium. To this view I wish to offer certain opposing facts obtained from a study of sponges. It has already been stated that no nervous structures have been definitely identified in the sponge, nor is there, so far as I am aware, any physiological reason to suppose that such exist. Nevertheless these animals are capable of some movements and their movements are so related to changes in the environment as to be classed as normal reactions. Under this head may be placed the closing and opening of the oscula and of the pores, and certain general movements of the whole body of the sponge. The closing of the oscula and of the pores is carried out by sphincters composed of spindle-shaped cells which in many respects resemble smooth muscle-fibers. These cells are unprovided with nerves and are brought into action, so far as I have been able to ascertain, by direct stimulation. I therefore believe them to be independent effectors and that the sponge is an example of an animal that possesses muscle but no nerve. If, as seems probable, muscle without nerve exists in these primitive metazoans, it follows that we are no longer justified in concluding that nerve and muscle have differentiated simultaneously, but it must be admitted that muscle is phylogenetically the older. I therefore believe that the beginning of the neuromuscular mechanism is to be found in the appearance of independent effectors such as muscles and that sponges probably represent this initial stage in the evolution of the mechanism concerned. Some physiologists may be inclined to question the actual occurrence of normally independent effectors, but the heart of *Salpa*, and that of the chick embryo before it becomes invaded by nervous tissue are examples of this kind, and it is now well known that though the sphincter pupillae of the vertebrate eye is under the control of nerves, it also responds directly to light. These instances seem to me a sufficient warrant for a belief in the existence of independent effectors.

Although the sphincters of sponges are effectors without nerves,

they are good examples of the kind of centers around which nervous tissue probably first arose. This development can be conceived to have occurred in the epithelial cells in the immediate proximity to such a center, in that these cells gradually assumed a special receptive function whereby they could stimulate the adjacent muscle more efficiently than it could be stimulated directly and thus an ordinary epithelial cell would gradually be converted into a receptive or sense-cell. From this standpoint the original function of the sense-cell was merely that of a delicate trigger by which the muscle would be more certainly and efficiently brought into action than through its own receptive capacity and many sense-cells in the lower metazoans probably still retain this as their sole function. Such cells occur abundantly in the coelenterates and hence I regard the sense-cell as the first type of nervous tissue to be differentiated. Since sense-cells and muscle-cells make up the chief part of the neuromuscular apparatus of coelenterates, I have designated this apparatus as a receptor-effector system.

But coelenterates usually show more than a simple receptor-effector system, for the fine branches from their sense-cells not only reach their muscle-cells but also anastomose with one another and form a nervous net. Such a net is the first step toward the formation of a central nervous organ or adjustor and its origin in relation to the sense-cells and the muscle-cells is probably so strictly local that it practically realizes Hensen's view as to the histogenetic relations of nerve and muscle, namely that these elements are not developed separately and brought into connection secondarily, but that their connections are original and give evidence of the incompleteness of cell division in the course of ontogeny. Such nets serve as more or less diffuse transmitters and are supplemented by the fibrils from certain contained cells, the so-called ganglion cells, which have migrated into the net and which probably mark the first step in the growth of those accumulations of cell-bodies that characterize the central nervous organs of the higher animals.

If this view as to the mode of origin of the central nervous organs is correct, it follows that these organs must be controlled in their incipency by the sense organs. In such coelenterates as sea anemones where the sensory specialization is slight, there is scarcely

any evidence of centralization in the nervous net, but in jelly fishes where the sense organs are specialized and in groups, each group has associated with it a region of special development, an incipient central organ in the nervous net. In bilateral animals such as the annelids and the crustaceans, the chief portion of the central nervous system, the so-called brain, is also associated with a group of sense organs and these organs, essential to the anterior end of any animal that moves forward, determine, in my opinion, the position of the brain rather than the reverse. Even in the vertebrates where the brain arises to a dignity not attained by any other nervous organ, its anterior position has been determined, I believe, by the location of the sense organs rather than that the sense organs are at the anterior end because the brain is there.

But although the central nervous organs have probably developed from a nervous net under the influence of the sense organs, they have taken in their later evolution a course more or less their own. Even the nervous net, which, in my opinion, unquestionably exists in the lower metazoans, has been denied in the central nervous organs of the higher animals. But it is not impossible that in these more specialized forms a nervous net may have a local existence. Evidence that nervous nets do not exist in certain parts of the vertebrate nervous system does not prove that they may not occur in other parts of this system. In the myenteric plexus of the vertebrate intestine the relations of nerve and muscle are such as to recall most strikingly the conditions already portrayed in the nervous net and muscles of the coelenterates and it is possible that nervous transmission in these animals follows the same rules that it does in the vertebrate intestine. In the vertebrate retina, too, the histological evidence is strongly in favor of a nervous net and the fact that the cells of the retina are members of the same epithelial layer and may therefore always have retained primary connections, suggests a fundamental similarity with the conditions in the coelenterates. Thus there are localities in the nervous systems of even the most highly differentiated animals where these most primitive of central structures, the nervous nets, very probably occur. But to claim on the basis of these instances that the whole central nervous system of the vertebrate is con-

structed on the plan of a nervous net would be going far beyond the facts.

It is well established that in the histogenesis of the central nervous organs of the higher animals, many cells that are ultimately in most intimate physiological relations, are in their early stages of development far asunder and that they attain to their final close relations by throwing out processes that grow toward one another. It is probable that these processes never really unite into continuous transmitting tracts but retain at least a certain physiological separateness, for in such parts of the central organ where these relations occur, transmission is not diffuse, as in the nervous net, but is limited to a single direction. Central nervous systems having these peculiarities have been called synaptic because the contact points between their cells, the synapses, are believed to be the parts that in some way govern the direction of transmission. This synaptic system has in the higher animals replaced to a considerable extent the more primitive nervous net and though this nervous net may still exist in some parts of the central nervous apparatus of such animals as the vertebrates, it is not the structure that gives to these organs their distinguishing characteristic. In these organs the fully differentiated nerve-cell or neurone with its synaptic connections is the characteristic structural unit of the system. Combinations of such units make up large parts of the central nervous organs of the higher animals and possess apparently physiological possibilities of a vastly higher order than can be found in the more primitive nervous nets; they have thus afforded the structural basis for the nervous activities of all the higher animals. Although the nervous net with its capacity for diffuse transmission was the structure in which the central nervous system took its origin, I nevertheless believe that this system early underwent fundamental changes whereby synaptic neurones with transmission in restricted directions replaced in large part the more primitive system of diffuse nervous nets.

The facts briefly stated in the preceding paragraphs justify the conclusion, I believe, that muscular tissue and nervous tissue have not arisen at the same time phylogenetically, but that muscle in the form of independent effectors preceded nerve in its develop-

ment and that nervous tissue differentiated in close proximity to muscle tissue as groups of sense-cells or receptors. Still later central nervous organs developed between the receptors and the effectors, first as clusters of nerve or ganglion cells which added to the nervous nets and later as aggregates of synaptic neurones from which were formed the more complex nervous organs of the higher animals. Thus the three parts of the differentiated neuromuscular system of the higher animals have, in my opinion, developed in sequence: first, the muscle or effector; next, the sense-organ or receptor; and last, the central organ or adjustor.

## 2. THE RELATIONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS IN PHYLOGENY

C. JUDSON HERRICK

*University of Chicago*

WITH TWO TEXT FIGURES

The fundamental factors in the differentiation of nervous and non-nervous tissues have been clearly presented by Dr. Parker, whose researches have the great and rare merit of combining both anatomical and physiological view-points and methods.

I commented yesterday<sup>1</sup> upon the striking parallelism between the series of animals when arranged according to structure by the comparative anatomists and the series when arranged according to functional type by students of animal behavior, and I pointed out that the ventral segmented ladder type of central nervous system, as seen in annelid worms and arthropods, naturally by virtue of its structure expresses itself in rigidly predetermined or stereotyped instinctive behavior, while the dorsal tubular and imperfectly segmented nervous system of vertebrates is structurally adapted to serve both reflexes and instincts as in arthropods and also the more plastic individual reactions of the intelligent type. The pre-eminence of vertebrates in the ability to perform individually acquired intelligent acts not predetermined in the hereditary nervous pattern is due primarily, I maintain, to the mechanical advantages of the tubular nervous system as compared with the ladder type of nervous system in the elaboration of correlation tissue, and I wish now to illustrate this thesis somewhat more fully from the anatomical side.

Let us take as our point of departure a very simple metazoan

<sup>1</sup> The Evolution of Intelligence and its Organs. Address of the vice-president and chairman of Section F, Zoölogy, of the American Association for the Advancement of Science. *Science*, N. S., vol. 31, No. 784, pp. 7-18, Jan., 1910.



body with a differentiated head end, bilateral symmetry and a diffuse or very imperfectly centralized nervous system, such an animal, say as a turbellarian worm (Fig. 1), which habitually creeps upon the ground. Within the outer epithelium is a layer of locomotor musculature (*M*) and a central digestive tract (*G*) and between these the other organs of vegetative life. Outside impressions are received chiefly by contact stimuli; and the diffuse nervous system is concerned for the most part with these stimuli and with internal or visceral reactions. The dorsal epithelium (*SEN*) alone is exposed to any considerable number of stimuli from distant objects, such as light and heat rays, currents and vibratory disturbances in the surrounding medium, emanations of odorous particles, etc. This animal can respond to a very small number of such stimuli. If such a species continues to crawl upon

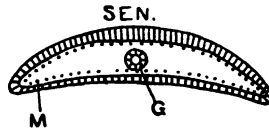


Fig. 1

or within the mud, after the manner of the worm-like ancestors of the arthropods, the contact receptors, especially those of the ventral and lateral surfaces, will in the course of evolution become more highly developed and the diffuse nervous system is naturally concentrated into a ventral central nervous system contiguous to these receptors. This vermiform locomotion and the associated transverse segmentation have in fact so firmly fixed the ventral ladder type of nervous system in the articulate phylum that even the free swimming crustaceans and the insects depart but little from it.

If, however, the hypothetical ancestral species with a diffuse nervous system assumes from the start a free swimming habit, this will tend to promote the differentiation of the dorsal distance receptors rather than the ventral contact receptors. That is, the

stimulation complex which reaches this free swimming body will contain a relatively smaller proportion of elements affecting the ventral and lateral body surfaces by contact and a larger proportion of elements emanating from distant objects and reaching the dorsal and oral surfaces.

Not only has such a differentiation of the dorsal epithelium undoubtedly taken place in ancestral vertebrates, but the primary correlation centers for these receptors have also been derived from the same source, and ultimately the correlation centers for the greater part of the contact and visceral reactions came to be incorporated with them, only the peripheral sympathetic system retaining the primary diffuse formation.

Sherrington has shown<sup>2</sup> that the mammalian cerebral cortex has been elaborated largely to serve the distance receptors; I think that we may carry the idea further back and say that the entire vertebrate central nervous system was from its earliest inception differentiated away from the annelid and arthropod type under the same influence.

A difference in habitual reaction to the common environmental forces on the part of a primitive animal with a diffuse and undifferentiated nervous system may, therefore, be said to have set the direction of two divergent lines of adaptation, one culminating in insects with (predominantly) instinctive action systems, the other culminating in primates characterized by individually adaptive and intelligent actions. The ultimate explanation for this divergence goes back, as in the case of all other evolutionary movements, to differences in the animal's reactions to the environment. Once the structural pattern has been thus laid down and fixed in the hereditary machinery, perhaps by natural selection, the future course of evolution is in some measure predetermined by the structural possibilities of the organs so differentiated. Thus, the ventral ladder type of nervous system favors the differentiation of an instinctive type of behavior based fundamentally on segmental reflexes, while the dorsal tubular type is better adapted for the development of longitudinally arranged correlation tissue which

<sup>2</sup> *The Integrative Function of the Nervous System*. New York, 1906.

facilitates total rather than segmental responses and a higher degree of integration of the whole system. Here I think we have clearly the machinery of a certain kind of determinate evolution which contains no elements of mysticism but rests on an intelligible basis of inherited type of nervous organization and action system.

Herbert Spencer's definition of life is biologically sound in that he makes the measure of correlation of internal with external forces the criterion of life. The lowly organism touches the environment at few points, receives but little from it and gives but little back. With the increase in the range of this effective contact with outer forces, the mechanism of internal regulation necessarily becomes more complex. Thus we have from the beginning of differentiation the somatic or exteroceptive activities set over against the visceral or interoceptive.

This finds its anatomical expression in two fundamentally distinct types of reflex arcs: (1) the somatic system, comprising the peripheral or exteroceptive sense organs, somatic sensory nerves and cerebral centers and somatic motor centers and peripheral nerves ending in the skeletal muscles, the whole system serving those reactions which the animal makes in response to external stimuli. (2) The visceral system, comprising the sense organs of the viscera, termed interoceptors by Sherrington, the visceral sensory nerves and centers and the visceral efferent centers and peripheral nerves, terminating in visceral muscles, glands, etc. The coördinating centers of the visceral system are partly peripheral in the sympathetic ganglia and partly in the central nervous system; those of the somatic system are wholly central.

In the central nervous system, then, we find evidences more or less clearly preserved of four fundamental longitudinal columns on each side of the body. These are so arranged that as one passes from the dorsal toward the ventral side of the neural tube in cross section he meets first the somatic sensory centers, then the visceral sensory, the visceral motor and the somatic motor. The relations of the four primary longitudinal columns of the central nervous system to the dorsal ectoderm will next be considered.

In all vertebrate embryos we find the dorsal nervous tissue at

the appropriate stage in the form shown in Fig. 2, the mid-dorsal epithelium being in process of invagination to form the neural tube. The figure is purely schematic and includes some features which are clearly differentiated only in later developmental stages. The somatic sensory surface (exteroceptors of Sherrington) includes both specialized sensillæ (*SEN*) and general sensory endings widely distributed in and under the epidermis. At the lip of the neural groove is the neural crest tissue (*N.C.*), from which the spinal ganglion cells will arise. These receptive cells sometimes, however, remain in the outer epithelium, either permanently, as in the olfactory organ, or temporarily, as in the gan-

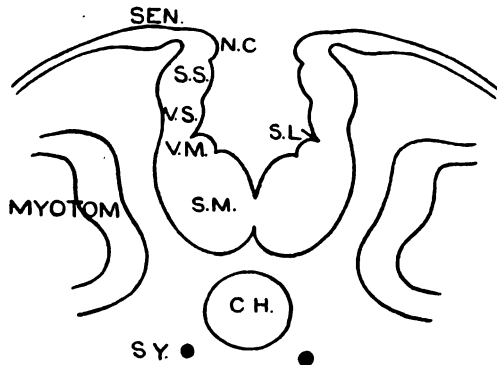


Fig. 2

glionic elements which are added to the cranial nerve ganglia from the embryonic cutaneous placodes. In other cases they are incorporated in the neural tube, as in the giant cells of the spinal cord of some fishes and the retina.

In the walls of the neural tube the dorsal part becomes the primary sensory centers, and separated from it by a very constant longitudinal groove, the sulcus limitans (*S.L.*) the ventral part becomes the primary motor centers. These are further subdivided, the somatic sensory centers (exteroceptors and proprioceptors of Sherrington) lying dorsally close to the neural crest and outer skin, the somatic motor far ventrally close to the myotomes and

the visceral sensory and motor between. These four primary functional columns can be more or less clearly recognized on each side of the neural tube in all vertebrates. This arrangement, while very different from that of arthropods, is *per se* no better adapted for higher psychic manifestations. But in later embryonic stages, to these primary centers there is added the correlation tissue of the reticular formation and the suprasegmental centers; and we have the key to the greater potentiality of the vertebrate type in the favorable form of the tubular nervous system, as contrasted with the ladder type, for the elaboration of this tissue.

The organs of somatic response in vertebrates are themselves so very complex as to require a special coördinating machinery of their own, such as muscle spindles, sensory endings of tendons, joints, etc. These, with their cerebral centers and return pathways, are termed by Sherrington the proprioceptive reflex apparatus. It is genetically and anatomically subsidiary to the exteroceptive system. Besides the sense organs within the somatic muscles, etc., mentioned above, this system includes the organs of the labyrinth of the internal ear and the associated cerebral centers of equilibration and muscular coördination. The cerebellum has been developed from the somatic sensory column of the medulla oblongata as the chief central coördinating apparatus of the proprioceptive system. The somatic system of reflex arcs is, accordingly, divided into exteroceptive and proprioceptive systems, whose receptors and cerebral centers are distinct, but whose efferent pathways are the same—to the somatic muscles in both cases.

The exteroceptors are further subdivided into contact receptors (organs of touch, etc.), and distance receptors (such as the eye and ear) the former being stimulated by objects at the body surface, the latter by forces emanating from distant objects. Evidently the type of reaction must necessarily be very different in the two cases.

The anatomical structure of the vertebrate central nervous system has been molded under the influence of two factors which have often been antagonistic. The first of these is the primary bodily metamerism, in accordance with which each segmental

At the Baltimore Meeting of the Anatomists in 1908, several anatomists interested in the study of human embryology decided that it is desirable to publish a list of the human embryos found in our various laboratories, in the ANATOMICAL RECORD. It is believed that this list will be not only of great value to those who are conducting studies in the anatomy of the human embryo but also will encourage others to collect embryos and make them available for scientific research.

The list now prepared includes those embryos in the principal collections, but it is desirable to make it as complete as possible. Those who have specimens which they wish to include in this catalogue are requested to send the data, as indicated below, to Dr. F. P. Mall, Johns Hopkins University, Baltimore, within the next few weeks.

List of Human Embryos in the Collection of .....

No. of Embryo.....	
No. of Slides in Series.....	
Crown Rump Length	
in millimeters.....	
Fresh.....	
Formalin.....	
Alcohol.....	
Clearing fluid.....	
On slide .....	
Remarks.....	
Direction of Section {	T. Transverse
	S. Sagittal
	F. Frontal
Thickness of Sections in microns .....	
Stains .....	
Condition of tissues {	E. Excellent
	F. Fair
	G. Good
	P. Poor



nerve tends to repeat exactly the same arrangement of components. This is far more evident in the lower vertebrates than in the higher, though it is never so important a factor as in the annelid worms and most articulata. This factor is more and more completely obscured as we ascend the vertebrate series by the second factor, viz., the longitudinal integration and correlation of the several functional systems which we have enumerated above. The more highly developed functional systems tend to be structurally more perfectly unified and concentrated, and this disturbs both the metamerism and the longitudinal patterns. Examples of this sort of disturbance are found in the tendency of all of the cutaneous nerves of the head to enter by the trigeminus and of the vagus to absorb the visceral components. In the rostral end of the brain the development of the massive suprasegmental correlation centers disturbs the primitive relations still more. But the primary pattern as we have outlined it is clearly evident in the structure of the medulla oblongata (either adult or embryonic) and its nerves in all vertebrates, and the comparative morphology of this part of the nervous system may be regarded as definitely established in its main features. The history of the steps by which this correlation has been effected would be an interesting contribution to scientific method.

After the formulation of Bell's law of the sensory character of the dorsal spinal roots and the motor character of the ventral roots, morphologists were long absorbed in the vain attempt to reduce the cerebral nerves to a similar simple segmental scheme. Even after Gaskell and His had laid the foundation for a true morphology of the medulla oblongata and its nerves, the deceptive simplicity of the older metamerism schemata still dominated the field and misled some of our ablest anatomists and embryologists.

As anatomists we have been slow to recognize the importance to our work of certain facts which have long been physiologically obvious. It was not until members of our own number, working with anatomical methods, brought out the structural pattern of the nervous system in some of the lower vertebrates where it presents almost diagrammatic simplicity, that we have directed our attention to them.



For four hundred years the cranial nerves had been dissected and for fifty years their central courses had been studied microscopically before any one succeeded in effecting a precise correlation of the peripheral with the central courses by following the nerve roots accurately through the ganglionic plexuses, and thus making an anatomical demonstration of the composition of the reflex arcs known physiologically to be there represented.

Acting under the stimulus of a suggestion made by Professor H. F. Osborn in 1888, a small group of American neurologists has patiently unravelled the tangled threads of the cranial ganglionic complexes in representative vertebrates, and now we are able to formulate a structural paradigm or type form of cerebral nerve components for the vertebrates as a class. The completion of the picture by the addition of further anatomical details, especially as to the corresponding central relations, by embryological studies and by physiological experimentation is rapidly progressing. This doctrine of nerve components, though first formulated in anatomical terms, is essentially a physiological conception, defining the peripheral and central pathways of the great fundamental types of reflexes, as I have endeavored to show by placing the emphasis on the physiological side and by the use, in this discussion, so far as possible, of the luminous terminology of Sherrington.

That the fundamental pattern of the vertebrate nervous system, as here laid down in terms of functional systems, is scientifically true is shown by the essential harmony of the data developed, for the most part entirely independently, in the fields of comparative anatomy, physiology and embryology. Conversely the most recent and perhaps the most striking illustration of the clarifying influence of these physiological units upon vexed morphological questions is given by the work of Landacre to be reported in this symposium.

The whole ectoderm of the vertebrate embryo, particularly on the dorsal side, must be regarded as potentially nervous. Part of this tissue is incorporated into the neural tube, a part is used to form the neural crest and peripheral neurones and a part develops sensory functions *in situ* peripherally. The relations of the peripheral receptive cells to the parent ectoderm are various. Prim-

itively these receptors were in the epidermis and some retain this position throughout the whole course of the phylogeny, as in the case of certain elements in the skin of annelid worms and of *Amphioxus* and in the vertebrate olfactory organ.

The general cutaneous innervation in lower forms is responsive to a considerable variety of stimuli. Even the human skin has several very different sensation qualities whose physiological analysis has proven very difficult, and it is still uncertain whether all of these qualities are served by specifically different nerve fibers or whether the analysis is in part central. The whole skin is very sensitive to chemical stimuli in fishes and in man it has been shown that general sensory nerves, not belonging to the gustatory system, are sensitive to certain chemical stimuli wherever they distribute to moist surfaces, as in the mouth cavity, though special end-organs, nerve components and central stations are differentiated for the more highly specialized chemical senses, taste and smell.

Parker has shown that the general body surface of lower vertebrates is also sensitive to light. But the great physiological importance of distance receptors of this type has led to a concentration of this function in special areas of ectoderm, the optic pits, which were involved in the invagination of the neural tube and finally again evaginated as the optic cups in order to bring the retinal surfaces into a peripheral position more favorable for receiving the light rays. That the retina is a modified somatic sensory receptor is confirmed by Johnston's demonstration of the close anatomical relationship in lower vertebrates of its most primitive cerebral center, the tectum opticum, with the general cutaneous centers. An interesting side light is also shed upon this question by Whitman's demonstration in 1892 (*Festschrift f. Leuckhart*) that in certain leeches *sensillæ* appear on each segment which in the caudal part of the body apparently function as organs of touch, but as we pass toward the head in successive segments, they become progressively modified in the direction of photoreceptors until in the head segments they are well formed eyes. Though this is probably a case of independent parallel differentiation, and is not ancestral to the vertebrate visual organs, it assists in the interpretation of the latter.

The ganglia of unspecialized sensory components of the peripheral nerves in general, both visceral and somatic, are derived from the neural crests, i. e., from masses of ectoderm at the lateral borders of the neural tube at the line of its separation from the general ectoderm. That this neural crest tissue is intermediate in type between the general ectoderm and the neural tube is shown by the fact already mentioned, that the ganglion cells of peripheral general cutaneous nerves are sometimes enclosed within the neural tube (the so-called giant cells of the spinal cord of some fishes) instead of lying in the usual position laterally of the spinal cord.

Evidence is constantly accumulating that some if not all of the special sensory components have been derived from the unspecialized visceral and somatic sensory components. The history of the evolution of the lateral line and auditory systems from the unspecialized somatic sensory systems may be regarded as demonstrated from the fields of comparative anatomy, comparative physiology and comparative embryology. The history of the central differentiation of the lateral line lobe and tuberculum acusticum from the somatic sensory column has been clearly demonstrated anatomically by Johnston; that the lateral line and auditory functions are closely related to the general tactile sense has been shown physiologically by Parker; and Landacre is able to illustrate in the embryological history of fishes an interesting relation between the neural crest and the dorso-lateral series of placodes in the origin of the lateralis and acoustic ganglia.

A similar history is presented in the visceral sensory system, where the ganglia of the unspecialized visceral nerves come from the neural crest, while those of the specialized gustatory component come from a special system of cutaneous placodes.

The olfactory nerve probably belongs to the last type, with this difference, that its peripheral neurones retain their positions in the placode instead of migrating inward to form a ganglion. There are, however, some elements which migrate from the olfactory placode to form a deep ganglion on the olfactory nerve, whose morphology is very obscure. Some of these migrating elements have been shown by Brookover in *Amia* to form sheath

nuclei of the olfactory fibers, others differentiate into the neurones of the ganglion of the nervus terminalis. The character of the latter nerve and ganglion demands further investigation.

Thus we find that each functional system of nerves has its peculiar type of development, peripheral end-organs, nerve components, ganglia and central connections, and for that the reflex arcs established among these functional systems constitute the most valuable units of nervous structure and function. Segmental and other gross subdivisions, which have the sanctions of long use and practical convenience, will of course continue to serve a useful purpose, but the fundamentally valuable data of neurology will more and more tend to be cast in the molds of these functional systems. This is true because in animal evolution the controlling fact has been the adjustment of the body to various environmental influences and the nervous system has been the medium of this adjustment.



### 3. THE ORIGIN OF THE SENSORY COMPONENTS OF THE CRANIAL GANGLIA

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WITH THREE FIGURES

Professor Herrick has set before us clearly the principal conclusions derived from the attempt to analyze from a functional standpoint the cranial and spinal nerves, chiefly among the Ichthyopsida. The writer will confine himself to the inquiry as to what support these conclusions find in the development in a favorable type such as *Ameiurus*, laying emphasis largely upon the mode of origin and the morphological relations of the cranial ganglia, exclusive of the sympathetic ganglia. The ganglia in the vertebrates are the source of the central and peripheral fibers which have been grouped into the various component systems, and are in a very literal sense the foundation of these systems. Their mode of origin must affect vitally our conception of the theory of nerve components. The cat-fishes were chosen as a type, partly because the nerve components of the adult are known through Dr. Herrick's work, and partly because the character of the gustatory system is such that it seemed to be a favorable form in which to differentiate between the special visceral and general visceral systems of ganglia.

In contrast with the favorable conditions offered by the embryo, the cranial nerves of the adult cat-fish are much more difficult to analyze than those of such a form as *Menidia*, but by going back to a stage between eighty and ninety hours after fertilization, we find the ganglia in such a simple condition, with so little fusion of the various ganglionic components, that their analysis becomes comparatively easy. At this stage (as shown in fig. 1) we find

with one exception that the various ganglia are arranged exactly as in *Menidia*. The exception is found in the case of the 9th nerve, which contains a special somatic or lateralis ganglion which is absent in *Menidia*. The visceral system (in horizontal shading, Fig. 1) is not differentiated into a general and special visceral system, but is left as shown in Professor Herrick's chart of *Menidia*.<sup>1</sup> We find here general somatic ganglia (unshaded in Fig. 1) in the

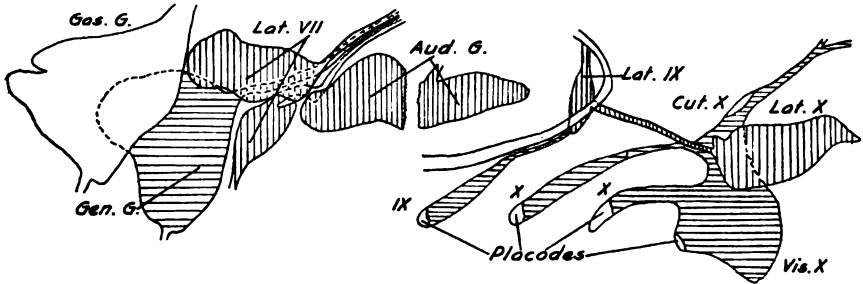


Fig. 1. Reconstruction of the cranial ganglia of *Ameiurus melas*. Oc. 8, obj. 4mm, Spencer. Trigeminal, facial and anterior half of auditory from an embryo of 86 hours. The posterior half of the auditory, the glossopharyngeal and the vagus from an embryo of 93 hours. General somatic ganglia unshaded; special somatic ganglia indicated by vertical shading; general and special visceral ganglia combined, indicated by horizontal shading.

5th or gasserian, and in the 10th. Special somatic ganglia (in vertical shading, fig. 1) supplying the ear and lateral line organs, are found in the 7th, 8th, 9th and 10th. General and special visceral ganglia are found in the 7th or geniculate in the 9th and in four divisions of the 10th. The general and special visceral ganglia, as mentioned above, cannot be separated in any type in the adult, and even in a late stage of development cannot be distinguished.

Starting with this type of ganglionic arrangement so common among the Ichthyopsida, let us inquire briefly how the various components are derived. In the region of the spinal cord, we have two components represented, the general somatic and the general visceral, both of whose ganglia are derived from the neural crest. In the head we have the general somatic and the general

<sup>1</sup> The cranial and first spinal nerves of *Menidia*. *Jour. of Com. Neu.*, fig. 3, 1899.

visceral components present also, and these are derived exclusively in *Ameiurus*, and probably in other types, from the neural crest, so that for these two fundamental systems the cranial ganglia and spinal cord ganglia fall into one category, as far as their mode of origin is concerned. This fact tends to emphasize in this respect the essential similarity of the head and cord region rather than the priority of one over the other. Whatever type of specialization the head region may have undergone — and the specialized ganglia furnish the same kind of evidence as that furnished by other structures — the two regions are essentially alike in these two fundamental systems, both of which are very old phylogenetically and quite generalized. We need not conclude, however, that because these two systems are old phylogenetically and generalized and are represented in both cranial and trunk regions, that they stand in any genetic relation to specialized systems of ganglia, such as the special somatic and special visceral ganglia of the head.

In the discussion of the relation between general and special ganglia, the chief interest centers about the mode of origin of the special somatic and special visceral ganglia, which are peculiar to the cranial region and are not represented in the trunk.

It will be easier to follow the origin of the special visceral or gustatory ganglia first. It has been known for a long time that certain of the cranial ganglia derived from the neural crest come into contact with the lateral epidermis in at least two regions. The more dorsal of these regions is at the level of the auditory vesicle, at which point the epidermal thickenings are known as dorso-lateral placodes; and the more ventral of these regions is at the level of the dorsal portion of the gill slit, where the epidermal thickenings are known as epibranchial placodes. The majority of observers, however, have expressed doubt as to whether the epidermis contributes cells to the neural crest portion of the ganglia.

In *Ameiurus*, owing to the hypertrophied character of the gustatory ganglia and possibly to the precocious appearance of these ganglia, there can be no doubt that epibranchial placodes do contribute cells to the neural crest ganglia. These placodes are not mere contact points, but are true epidermal thickenings which



proliferate cells medially so that they are added in most cases *en masse* to the neural crest ganglia, and there is little cause for confusion as to their true nature. This mode of formation of the gustatory ganglia can be determined easily during the growth of the embryo in the case of the 7th and in the first two divisions of the 10th ganglia. The strongest confirmation comes, however, in the case of the epibranchial ganglion of the 9th nerve. In this nerve Professor Herrick can find only one type of visceral fibers, the special or gustatory, and in the development of the 9th visceral ganglion the writer can find no trace of cells other than those that come from the placode, so that one is warranted in concluding that the special visceral or gustatory ganglia come from the epibranchial placodes in *Ameiurus*. Every ganglion giving rise to gustatory fibers is derived in part from the placodes. This is true of the 7th, 9th, and four divisions of the 10th ganglia. (These are shown in cross-hatched shading in Fig. 2.) The fourth placode

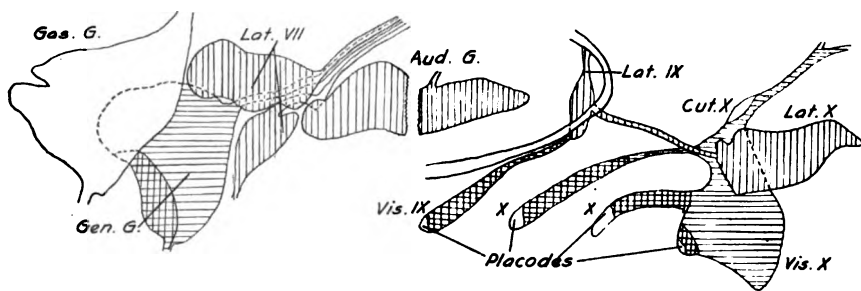


Fig. 2. Ganglia as in fig. 1, to show the origin of the special visceral components. General somatic ganglia unshaded; special somatic ganglia indicated by vertical shading; general visceral ganglia indicated by horizontal shading; special visceral ganglia indicated by cross-hatched shading.

of the 10th ganglion had not appeared at the age at which this reconstruction was made. Further than this, the placodal derivative in each ganglion is in a general way proportionate to the number of gustatory fibers coming from the adult ganglion.

The last two placodal derivatives of the 10th ganglion are quite small, and the last one answers quite accurately to many of the descriptions of the epibranchial placodes in the literature. The last placode of the 10th does not appear until after the neural

crest ganglion comes into contact with the skin, and is in fact indicated only by the presence of this contact. If it were not for the characteristic relation presented by the 9th nerve and the almost equally characteristic relation shown by the first two divisions of the 10th, one could easily accept for the last division of the 10th, the usual description in regard to the relation of the placodes to the neural crest cells, namely, that the neural crest comes into contact merely with the epidermis. But with the history of the 7th, 9th and the first two divisions of the 10th, we must conclude that the usual description answers to a condition where the gustatory component in a ganglion is small or late in appearance, as in the mammals and in man particularly, and that probably the placodal contribution to the ganglion is not sufficiently large or sufficiently well defined to be isolated, and does not appear before the contact is formed between the neural crest ganglion and the epidermis.

We have then in *Ameiurus* evidence that the special and general visceral systems, which have been separated in the adult on the basis of the difference in the peripheral distribution and type of fibers, can be isolated in the embryo on the basis of mode of origin of the two types of ganglia. The special comes from the epi-branchial placodes and the general from the neural crest. While this analysis of the ganglia cannot be made in the adult or even in a late stage of embryonic development, still the fact that they can be isolated in the earlier stages of their formation furnishes a striking confirmation of the analysis effected in the adult and tends materially to strengthen the point of view on which this analysis was made.

Turning now to the other special system of the head, the special somatic or acustico-lateralis, we find a somewhat different history with a rather sharp distinction between the mode of origin of pre-auditory and post-auditory components of this system. The last one of the series, the lateralis 10th, is derived exclusively from a dorso-lateral placode which is a posterior extension of the auditory vesicle. It becomes detached from the epidermis much as the epi-branchial ganglia do and contains no neural crest cells. The next two in the series, the lateralis 9th and the auditory, seem to come exclusively from the auditory vesicle but ow-

ing to the congestion of structures in the auditory region caused by the rapidly developing vesicle, it is difficult to be certain of their purely placodal origin. They may possibly contain neural crest cells in *Ameiurus* and have been described by other authors as arising largely from the neural crest. If they do contain neural crest cells, they represent a transition between the lateralis 10th, which is a pure placodal ganglion, and the condition to be described in the lateralis 7th ganglion. Passing anteriorly, we come to the first one of the series, or rather the first two, the lateralis ganglia associated with the geniculate ganglion of the 7th, which is

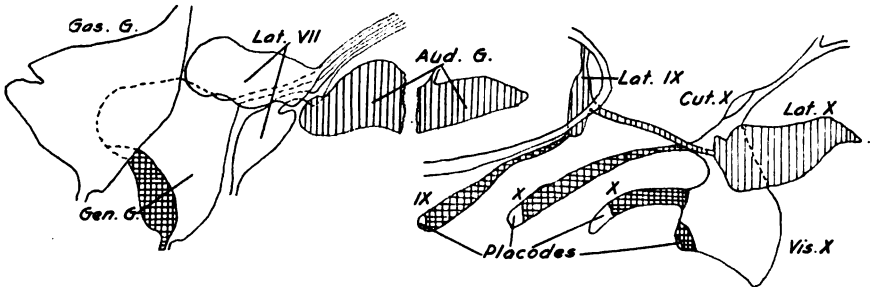


Fig. 3. Ganglia as in figs. 1 and 2, shaded to show source of origin. Unshaded ganglia derived from neural crest; derivatives of dorso-lateral placodes indicated by vertical shading; derivatives of ventro-lateral placodes indicated by cross-hatched shading.

pre-auditory in position. These two ganglia are derived exclusively from the neural crest and are totally unlike the lateralis 10th in their mode of origin.

We have thus as derivatives of the neural crest in the head all of the general visceral and general somatic ganglia of the 5th, 7th, 10th and the two special somatic ganglia associated with the geniculate ganglion of the 7th nerve. This is in rather striking contrast with the specific mode of origin of the special visceral ganglia, which are all derived from placodes, and at first glance seems to militate against the specific character of these ganglia as observed in the adult. But if one recall that the only condition imposed upon the nervous system, both peripheral and central, is that it shall furnish such correlations as will be profitable to the organism in adjusting it to its environment, it is not surprising

that sharp morphological distinctions should be rather frequently broken down. As illustrations of this, one has the dominance of certain centers in the brain as compared with the adjoining centers of similar characters: the usurpation by one nerve, of peripheral areas usually innervated by a totally different nerve and even by a different kind of component, or the dominating of a pure lateralis nerve such as the hyomandibular by components such as the general cutaneous and the visceral.

From a functional point of view, there is nothing unusual in the double mode of origin of the special somatic or lateralis ganglia of the head. As Professor Herrick has pointed out, the whole ectoderm of the head, particularly the dorsal and the lateral portions, is to be considered as potentially nervous. This is evidenced by the formation of the neuro-epithelium of the olfactory organ, by the formation of the ganglia from the epibranchial placodes and the dorso-lateral placodes, and by the formation of the neural crest ganglia and even of the cord itself. All the diverse modes of ganglion formation seem to serve equally well in connecting the peripheral sense organs with the central nervous system.

The olfactory neuro-epithelium, with its sense cells acting as ganglion cells also, is evidently the simplest type of vertebrate ganglion. Next come the optic ganglionic cells which, while derived from the brain wall, still are only slightly removed from their epithelial origin. These ganglionic cells remain in the nervous layer and retain their position near their sense cells and do not migrate into the mesoderm. Following this, one has the auditory ganglion derived from the auditory pit but moving into the surrounding tissue and away from its sense cells. Its primitive character is evidenced by its bipolar ganglion cells and by the fact that its placode still remains a sense organ. Next, there is the lateralis ganglion of the 10th nerve, which comes from a placode but becomes entirely detached from it and lies in the mesoderm. In *Ameiurus* this placode, unlike the auditory placode, does not become a sense organ. It may possibly do so in other types, as Wilson maintains, and it would then resemble the auditory ganglion. Following this there are the epibranchial ganglia derived from

placodes, concerning which there is no evidence in the ontogeny that these are sense organs at all. It seems probable, however, that in phylogeny they may have been derived from, or at least associated with, sense organs located at the dorsal portion of the gill slit, where the placodes now arise. If the epibranchial ganglia are included in this list tentatively, the whole class is characterized by the fact that they are associated more or less closely with special sense organs, which they afterward bring into contact with the brain, and thus serve to adjust the organism to its environment.

The neural crest ganglia stand in rather sharp contrast with this large class of ganglia, in that they are not associated in origin with any type of special sense organ and conserve the functions of general sensibility rather than the special senses, except in the case of the lateralis 7th ganglia, so that they can hardly be placed in a series with the first class. If the lateralis 7th ganglia were derived originally from the placodes in *Ameiurus*, as they seem to be in Cyclostomes, and have changed from a placodal type to a neural crest type, it is rather a process of usurpation than of evolution.

The pre-auditory cranial region presents other modifications as compared with the post-auditory region fully as remarkable as this. The writer believes that the specialized ganglia should not be considered as derived from the unspecialized ganglia in the sense that Johnston has shown the specialized centers of the brain to be derived from the unspecialized centers. These stand in a genetic relationship to each other. On the other hand, the placodal ganglia have arisen in the potentially nervous ectoderm in response to the need of a more definite correlating apparatus and have come from the region of the sense organ. The neural crest ganglia have arisen in response to the same need, but have come from the region of the cord and brain. In the case of the pre-auditory lateralis ganglia, the second type seems to have usurped the place and function of the first type and this may be going on even in the auditory and lateralis 9th. This change would be no more remarkable than other well known changes in the peripheral distribution and composition of nerves, which tend to adapt

the organism more accurately to its environment, or to a different environment, or to integrate the activities of the organism itself. All of these changes, while presenting puzzling morphological conditions, tend to emphasize the idea that the functional needs of the organism rather than consistency in morphological detail is the key to the complicated nervous mechanism of the vertebrates.

The original characterization of a nerve component included the following distinctive points. The peripheral sense organs are of a common type with a common function. The fibers of a given component are usually of a definite size, thus enabling one to trace them through their ganglionic connections to the brain. The ganglia are definitely localized and sometimes in the most favorable types sharply isolated from the adjoining ganglia. The central ending of a given component is definitely localized. From the embryological evidence, we can add to this characterization the fact that the ganglionic components are definite in their mode of origin and except in the case of the preauditory lateralis ganglia are specific in their mode of origin. The general visceral and general somatic ganglionic components represented in both cranial and trunk regions are derived from the neural crest. The special visceral components are derived from the epibranchial placodes. The special somatic components show a transition from a pure placodal type in the lateralis 10th through a possible intermediate type in the lateralis 9th and auditory, to the pure neural crest type in the lateralis 7th.



#### 4. THE PROBLEM OF THE CORRELATION MECHANISMS

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WITH ONE FIGURE

The next general problem before the anatomist of the nervous system is that of the correlation mechanisms. The organization of the primary receptive and effective mechanisms has found adequate expression in the doctrine of the functional divisions of the nervous system. The validity and the usefulness of this doctrine are demonstrated by its adoption by an increasing number of workers in this country and abroad. The task of elaborating a complete functional morphology of the nervous system, however, has only been begun by the theory of functional divisions.

The problem of the correlating mechanisms is as many-sided and complex as the nervous system itself, as broad and varied as the whole of human life. The problem involves (1) all those questions relating to the structure and connections of the individual neurones, the character of the nerve impulse and the mode of its propagation through the neurone and from one neurone to another; continuity, synapses, stimulus threshold, summation, inhibition, etc.; and (2) all those questions regarding the means by which simple reflexes are combined into larger actions directed for the welfare of the organism as a whole.

It is to the solution of the second set of problems that comparative neurology can contribute most at the present time. The problem is fundamentally more than all else a problem in the genesis of structures functioning in an adaptive manner. Structure and function can not be separated and both must be studied in the light of their purpose. Structures in action—actions performed by definite structures—have for their end the adaptation of the organism to the conditions of its life. The way in which the parts



of the nervous system work together in directing the various organs for the welfare of the organism as a whole is the chief guide in the interpretation of the nervous system. This has been the burden of the teaching of the functional morphologist. But to discover how the parts of the nervous system work together it is necessary to inquire how the nervous mechanisms have come to be what they are through the process of evolution of the race. What were the first or simplest structures serving certain functions? How were they modified and specialized? What causes led to the increasing complexity of their structure? By what steps have they come to be what they are? In this way alone can we discover fully what they are and what they mean. For this way of looking at the nervous system we may use the name *genetic method*. It is in attacking the most complex problem that a complete genetic method is most needed. The study of the correlating mechanisms, to repeat, is a study of the evolution of nervous structures functioning in such a way as to secure the adaptation of the organisms concerned.

How have simple reflexes been combined into adaptive action-systems? What are the impulse pathways by means of which one simple reflex is combined with others into a larger whole, while certain others are left passively idle and still others are actively shut out from participation because antagonistic to the main purpose? Nearly everyone of our common actions answers to this description, and those actions have grown up through a long past by the combination of simpler elements. We see this in the growth of the infant, in his learning to see things, to grasp objects, to walk, to talk; and we can more or less fully trace the phylogenetic history of some of our actions. The most direct and effective method of attack is to study the genesis of the actions themselves and the parallel genesis of the nervous mechanisms concerned in them. An excellent example of the right mode of approaching these problems is the study of the genesis of movements in amphibian tadpoles presented to this Association at its last meeting by Professor Coghill.

Among the many phenomena requiring explanation, the spread of reflexes to distant segments and the coöperation of distant seg-

ments in a single action will illustrate the point of view here suggested. Perhaps the most constant result in experiments upon animals is the tendency for the responses to be complete or partial reproductions of habitual or common acts. In the highly specialized reflexes of the dog certain kinds of stimulation produce definite movements. For example, stimulation of the shoulder in any of several ways calls forth the scratch-reflex. It is frequently noticed, when the limbs are called into action by a stimulus, that the form of their movement is dominated by the method of progression characteristic of the given animal. Thus in the dog various forms of excitation produce attitudes of the limbs which are due to the dominance of the trotting gait in this animal. If the stimulation be at a hind foot the movement of the fore leg or legs is that which would form part of the act of trotting. If painful stimulation of one hind foot in the spinal dog be continued the flexor muscles of that leg contract and the other three legs move in the rhythm of progression, that is, the hurt foot is held up and the other three feet run away (Sherrington, *Integrative Action*, p. 240). So, in experiments on lower vertebrates in which general somatic nerves are stimulated, the responses are movements of swimming. Witness the recent work of Sheldon on chemical stimulation of the dogfish. If the stimulation is strong enough it calls forth contraction of muscles of distant segments, perhaps of all segments of the body. In this spread of reflexes to distant segments we have one of the fundamental elements in the combination of reflexes.

The responses called forth by irradiation to distant segments through the spinal cord are not hap-hazard, but are parts of typical actions. The phenomena of irradiation must therefore rest upon systems of nerve paths produced in the course of the evolution of characteristic behavior of the given species. Long spinal irradiation is but a specific illustration of what we may call segmental, or *metameric correlation*.

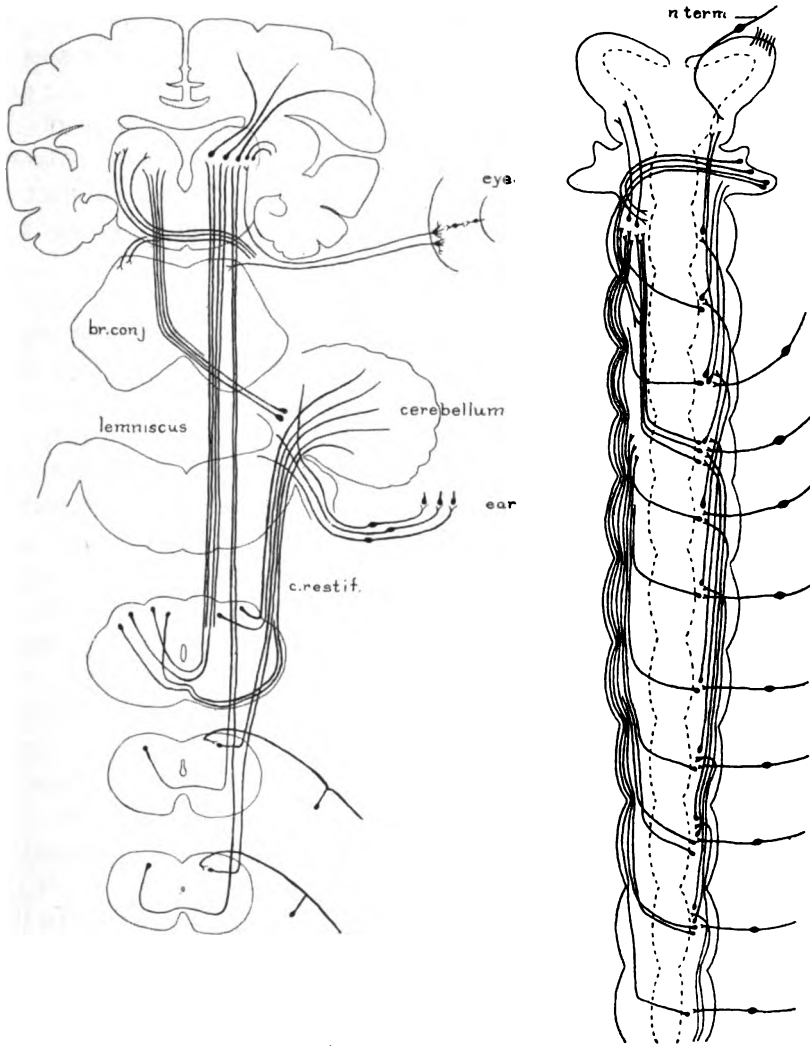
When we look for the mechanism of this segmental correlation, we find a plethora of materials and our difficulty is to sift out definite structures and discover their specific functions. In the spinal cord and brain of vertebrates we recognize in each of the

receptive columns, somatic and visceral, primary receptive neurones and other neurones (*substantia reticularis*) which constitute the structural means for correlation. Fibers arise from the somatic receptive column—the dorsal horn—to go to other segments to end in the same column on the same or opposite side. Other fibers go to the somatic motor column of one or more segments. These latter may serve to call into action larger masses of muscle, but can have only a low value in correlation.

Those neurones by which the in-coming impulses are spread to distant segments of the same column are of especial significance in correlation. Two chief sets of such neurones are recognized. The fibers of one of these run up or down the cord in proximity to the dorsal horn itself into which the fibers turn after a longer or shorter course. The second set of neurones send their fibers by way of the ventral decussation of the cord to end in the dorsal horn of the opposite side in a higher or lower segment.

Whatever may be the specific mode of functioning of the correlation neurones within the spinal cord, numerous long fibers of both sets of neurones have played an important part in the evolution of the brain. Homolateral fibers from the dorsal horn of the cord reach the cerebellum and many more join these from the nuclei cuneatus and gracilis of the medulla oblongata. These direct cerebellar fibers are joined also by external arcuates from the other side of the medulla oblongata. Crossed fibers from the dorsal horn of the cord together with many more from the nuclei in the oblongata, including the centers for the fifth and eighth nerves, and still others from the dentate nucleus of the cerebellum, form a great system or several systems of fibers, of which the medial lemniscus is the type. The fibers of both direct and crossed systems pass from the somatic sensory column of lower segments to the same column in higher segments. These are fundamentally segmental correlation fibers, but their great numbers and their definite arrangement with reference to certain segments and nuclei in the brain are due to a special significance which they have obtained in consequence of the development of special sense organs.

It is customary to speak of the development of organs of special sense in the head as the cause of the development of the brain.



SCHEMA OF SEGMENTAL CORRELATING TRACTS

On the left are shown some of the long ascending tracts concerned in somatic receptive functions in man. On the right is shown the segmented brain of a hypothetical primitive or ancestral form in which the evagination of the forebrain and the retinal areas has begun. Otherwise the brain is simply the anterior portion of the neural tube. In the arrangement of the direct and crossed correlating fibers the specialized tracts of true vertebrates are foreshadowed. The place of ending of the nervus terminalis in this figure is the primordial somatic cortex.

This is true, but we are too apt to lose sight of the importance of the correlation of general somatic sense organs with the special sense organs. The primary receptive centers for the eye, ear and nose account for only a small part of the increased size of the anterior part of the neural tube. The greater part of the enlargement called the brain is due to the material serving for correlation between eye, ear, and nose and between those and the skin and muscles of the body.

The mechanism for correlation of the general bodily organs with the organs of special sense was ready-prepared before those sense organs made their appearance in the vertebrate ancestors. The ancestral forms had no head, only an anterior end. What is now *head* was in those ancestors a region with a complete series of segments and sensory nerves. We have no evidence of somatic motor nerves further forward than the present oculomotorius. Two forms of sensory neurones were present: one which we may call the ganglion-cell type, sensitive especially to mechanical stimuli, with chemical, photic and thermal sensitiveness in the background; the other which came to form the olfactory organ, with chemical sensitiveness dominant. Both these had been derived perhaps from a single still more ancient and unspecialized type of peripheral sense cells. The specialization of the sense cells in the anterior segment of the body as cells of chemical sense and their collection into a restricted area gave rise to the first special sense organ, the olfactory. The sense cells of the rest of the body likewise collected into a long strip at either side of the neural plate and gave rise to the spinal and cranial ganglia. In this simple animal the chief long paths in the nervous system were concerned with segmental correlation and these paths form the basis for the high development of correlation mechanisms, which is the chief characteristic of vertebrate animals and which enabled this phylum, by adapting itself to wider and wider ranges of environmental conditions, to become the dominant race of animals.

A certain part of the ganglion-cell type of sensory neurones, especially in the anterior end of the body, from the first tended to specialize in the direction of light percipient cells and in three or more segments of the head these cells became aggregated into *eyes*.

These formed parts of the brain wall and became evaginated, as is well known. I have several times brought forward evidence that the eyes were developed from the general somatic receptive column and I have suggested that the optic tract fibers constituted essentially a correlation tract comparable to the lemniscus. The most important result following from these facts is now to be pointed out, namely, that this optic tract entered the somatic sensory column where its impulses came at once into relation with impulses from the skin and muscles, brought up by the long tracts for the sake, originally, of segmental correlation. Thus early the primordial structures were present which provided against the dominance of direct and unmodified reflexes, such as obtains in invertebrates, and provided for the control of body movements by the coöperation of two or more sensory mechanisms taking account of different factors in the environment. This it is which distinguishes all vertebrates from invertebrate forms,—the degree to which the power to guide their actions with reference to impulses of two or more kinds is developed.

When later, the acustico-lateralis system of sense organs arose to take account of slow wave-stimulation and developed into an organ of the static sense, and still later gave rise to an organ of hearing, these organs sent their impulses into the same somatic sensory column, whose long tracts served also for correlation of these with the skin and muscles.

It was in this way that the brain came to be developed as a great collection of correlation centers. The gray matter in the tectum and the thalamus, as soon as the eye was formed, served at once, not as optic centers alone, but as somatic-optic correlation centers. It is noticeable that the fishes which present well formed optic centers in the thalamus are not alone those with large eyes but the strong-swimming, active forms. For example, among ganoids the active and predacious freshwater dogfish (*Amia*) has a well developed lateral geniculate body, while the sluggish bottom-feeding sturgeon has not.

Again, the gray matter in the segments following the tectum became a center for the correlation of canal-organ impulses with those of the muscle sense in the control of muscular move-

ments. Here was developed the most sharply specialized and highly characteristic region of the vertebrate brain, the cerebellum, Deiter's nucleus and area acustica serving as a static mechanism, an organ for muscle tone, etc. The great importance to this mechanism of the sensory impressions from the muscles and the skin which are carried up by the dorsal tracts and restiform bodies, has been so often pointed out that we need not dwell on it here.

Further, the common and primitive basis of correlation tracts which put these sense organs into relation with the muscles and skin, served also to put them into relation with one another. This must be passed over for the sake of discussing briefly the conditions determining the development of the somatic cortical centers in which correlation of all these sense surfaces is brought about, and apparently on a higher plane.

The cerebral cortex consists essentially of two parts, a visceral cortex and a somatic cortex. The former will be discussed in another paper. Here let us examine briefly the conditions for the development of the somatic cortex. In the more active lower vertebrates the optic-somatic correlation centers play so important a part in the more intelligent seeming activities that some one has said that the tectum plays the part of cortex for the fish. Why have not some of the lower correlation centers, say the optic, developed into the cortex? Chiefly for the reason that the presence of the special sense organ demands the use of the greater part of the substantia reticularis in those centers for the direction of simple or combined reflexes *in which the impulses from one sense organ play a dominant rôle*. It is characteristic of cerebral cortex that it is free from the domination of any one kind of sensory impulses. Since there is some limit to the development of any correlation center—at least the limit of the power of growth with which that part of the nervous system is endowed in the embryo—a center which is largely concerned with any one sense could not well supply the material for cortical functions.

An influence favoring the development of cortical centers in the telencephalon is the presence of the olfactory centers in that segment. The olfactory organ is not only a special organ of the chemical sense of ancient standing, but it has acquired special

importance by reason of its power to function at a distance. As pointed out by Sherrington, the olfactory organ is a distance receptor in the search for food. It is important, therefore, that the olfactory organ be correlated with the visual organ and with the muscles which are chiefly concerned in the capture of food. Where is this correlation provided for? In part at least in the olfactory centers in the hypothalamus and epithalamus and the optic centers with which these are inter-connected. Indeed, these so-called olfactory centers in the diencephalon are in reality the meeting-places or clearing-houses for impulses of different sorts and should be called olfacto-gustatory, olfacto-visual and olfacto-muscular correlation centers. I see no reason why these centers should not have sufficed for the combination of all sorts of reflexes in which the olfactory organ was concerned as a distance receptor in the search for food. The most that we can say as to the influence of the olfactory organ is that an olfactory-somatic correlation center in the telencephalon would perhaps have some advantage in efficiency. The presence of the olfactory organ does not give any clue as to how such a center in the telencephalon came to arise.

For this we must turn to the principle of metameric correlation. The long correlation tracts are believed to be more fundamental and of earlier origin than the special sense organs or the brain itself, and if such tracts reached the first brain segment regardless of the olfactory organ, then the development of an olfacto-somatic correlation center in the telencephalon is merely a question of its usefulness to the organism. Was there present in the first segment of the neural tube of vertebrate ancestors a segment of the somatic sensory column? Was this connected with lower segments of the same column by metameric correlation tracts? And could such a center offer the material and the conditions for the development of the somatic cortex? I believe all these questions are to be answered in the affirmative.

There is connected with the forebrain in selachians a nerve, evidently vestigial, which bears a ganglion and is distributed to the epithelium of the nasal sac. I believe that this represents the general cutaneous nerve component of this segment. The nerve



enters a part of the forebrain which in selachians receives fiber tracts from lower segments of the somatic sensory column, namely, the lemniscus center in the thalamus and perhaps other centers. Here are evidences of the existence of a primary somatic sensory center and of correlating tracts in one of the lower groups of fishes. That ancestral vertebrates possessed a cutaneous nerve in the first segment and that its center was connected by long tracts with lower centers of the same sort is a reasonable deduction from this evidence and also is *a priori* very probable.

Such a center was very favorably placed for the development of somatic cortex for two chief reasons. First, it had the advantage of proximity to the olfactory centers and the olfacto-gustatory cortex. Second, the correlating material of this segment of the somatic sensory column was the only one to be set free from the dominance of a special sense organ; eye, ear, skin, or muscles. The N. terminalis disappeared and the cutaneous surface which it supplied was invaded by the trigeminus. The substantia reticularis of the forebrain center, was then released from the work of combination of simple reflexes and came to serve for correlations of a higher order. This is a special case of a general tendency in the brain which has long been recognized, namely, the tendency toward segregation and condensation of centers for special functions. The cutaneous innervation of the head, originally provided by some ten or eleven segmental nerves, is in man almost all provided by the trigeminus with some branches from the first and second spinal nerves, and its center is condensed into the medulla oblongata. The special sense organs were restricted to one or a few segments from the first and have dominated those segments, as we have seen. The forebrain segment of the somatic column, while losing its primary sensory function, offered the opportunity for olfacto-somatic correlation and for the inter-correlation of somatic organs which sent impulses up to it over the long tracts. It can not be thought that the occasion or impulse for the development of this correlating center after it was freed from its primary sensory function was supplied by the olfactory organ and centers alone. Olfacto-somatic correlation in the forebrain is to be regarded rather as incidental. Had there been no other occasion

for a somatic center in the forebrain, olfacto-somatic correlation would all have been cared for in the diencephalon. The cerebral cortex serves for correlation between tactile, muscular, static, auditory and visual impulses in a thousand ways in which olfactory impulses are not at all concerned. For the development of these somatic correlating functions the olfactory apparatus could have been neither the stimulus nor the directing force. If there had been no somatic sensory center in the forebrain, the somatic cortical functions would never have been located in the telencephalon. The determining factors in the development of the somatic cortex were: (1) the center for the *nervus terminalis* with the *substantia reticularis* belonging to it; (2) the fundamental correlation tracts bringing up tactile, musculo-sensory and visual impulses to this center; (3) the reduction of the nerve which left the *substantia reticularis* free to serve for correlation of the impulses just mentioned; (4) and the advantage of a center where impulses of different kinds might interact upon equal terms. In this last, which seems at first a vague and intangible principle, lies the very essence of the conditions for the development of the higher cortical functions, memory, judgment, reasoning and the aesthetic faculties. Consciousness springs, as I believe, from the tension of indecision between two or more sets of impulses, any one of which coming alone would be followed by a simple reflex; or between two or more possible responses to a stimulus. If so, we can not expect a very high grade of consciousness in animals in whose nervous systems each center is under the dominant influence of one sense organ. The tension in the olfacto-visual, olfacto-gustatory, or visuo-muscular correlation centers would, too often, be dissolved by the dominant influence of one or other sense organ. Inhibition would not be very prolonged, one set of conditions would not hold the attention long for the purpose of weighing the different impulses or responses over against one another. The solution of the tension through a simple reflex or a combination of reflexes of a low order or of a habitual type would be unfavorable to the development of memory, of adaptability in responses, or of deliberation, which is essential to intelligent action.

In the forebrain center, however, just those conditions are presented which are favorable to the development of the faculties of intelligence. In addition to the freedom from the unequal influence of one set of impulses, the fact that impulses reach this center only by long paths and usually by a relay of three neurones is of great importance. In the definition of cortex in general I have elsewhere given weight to the relay of three neurones for these reasons: (1) Such a relay removes the cortex farther from the realm of direct reflexes by increasing the *time* of reaction through the cortex. The cortex is never involved where extraordinarily quick response is necessary. (2) The relay restricts the number of impulses passing to the cortex. Impulses to reach the cortex either must have sufficient energy to command the right of way (through the synapses) or they must find the way prepared for them through *attention*; and attention itself is a conscious process and one of the greatest factors in the further development of consciousness.

If we were to look at the visceral sensory mechanisms we should find essentially the same arrangements as have been described for the somatic: a longitudinal column with long tracts connecting distant segments with one another. Into this column came the fibers of taste and smell and the long tracts brought these into relation in the forebrain, so giving rise to the visceral cortex.

I present, then, as three matters of great importance in the study of the correlation mechanisms: (1) the fundamental character of metameria correlation; (2) the development of the brain through the local hypertrophy of this segmental mechanism under the influence of the special sense organs, and the related segregation of special centers, and (3) the indifference of the somatic correlation center in the telencephalon, which offers the essential condition for the development of the cerebral cortex as the organ of conscious life.

# PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

## TWENTY-FIFTH SESSION

*In the Embryological Laboratory, Harvard Medical School, Boston, Massachusetts, December 28, 29, and 30, 1909.*

TUESDAY, DECEMBER 28, 9.30 A. M., TO 1.00 P. M.

The twenty-fifth session was called to order at 9.30 am. by President, James Playfair McMurrich, who appointed the following committees.

*Committee on Nominations*; CHARLES S. MINOT, *Chairman*; THOMAS G. LEE, SIMON H. GAGE.

*Auditing Committee*; MILTON J. GREENMAN, *Chairman*; AUGUST G. POHLMAN.

### SYMPOSIUM OF COMPARATIVE NEUROLOGY:

GEORGE H. PARKER, *Harvard University*. The phylogenetic origin of the nervous system.

C. JUDSON HERRICK, *University of Chicago*. The relations of the peripheral and central nervous systems in phylogeny.

FRANCIS L. LANDACRE, *Ohio State University*. The origin of the sensory components of the cranial ganglia.

JOHN B. JOHNSTON, *University of Minnesota*. The problem of correlation centers and the evolution of the cerebral cortex.

The general discussion was opened by Henry H. Donaldson, Wistar Institute of Anatomy.

The remainder of this session was devoted to the presentation of the following neurological papers:

STEWART PATON, *Princeton, New Jersey*. Neurofibrillation in relation to the first movements of vertebrate embryos.

SUSANNA PHELPS GAGE, *Ithaca, New York*. A pair of dorsal cerebral sacs on either side of the terma in a 35 day and other human embryos, comparable with the cerebral sacs in fishes.

S. WALTER RANSON, *Northwestern University Medical School*. Non-medullated nerve fibers in the spinal nerves.

HENRY H. DONALDSON, *Wistar Institute of Anatomy*. On the percentage of water in the central nervous system of the albino rat. (Lantern slides.)

S. HATAI, *Wistar Institute of Anatomy*. Preliminary report on the inheritance of the weight of the central nervous system in rats.

JOHN B. JOHNSTON, *University of Minnesota*. Early stages in the evolution of the cerebral cortex. (Only an abstract presented.)

The following neurological papers announced were read by title:

C. JUDSON HERRICK, *University of Chicago*. The analysis of the paraterminal body and its relation to the hippocampus in lower brains.

BURT G. WILDER, *Cornell University*. The weight and form of the brain of some American negroes; illustrated by specimens, photographs and charts.

ELIZABETH H. DUNN, *University of Chicago*. Some findings regarding the distribution of splitting medullated nerve fibers in the peripheral nervous system.

TUESDAY, DECEMBER 28, 2 TO 5 P. M. DEMONSTRATIONS AS FOLLOWS:

CHARLES R. ESSICK, *Johns Hopkins University*. (a) Specimens showing the development of the arcuate nuclei in the human embryo; (b) Dissections to show migration of cells in the medulla of the pig embryo.

SUSANNA PHELPS GAGE, *Ithaca, New York*. Models of the head of a five-weeks human embryo.

CLARENCE M. JACKSON, *University of Missouri*. Models of the thoracic and abdominal viscera of the human embryo.

JOHN B. JOHNSTON, *University of Minnesota*. Models illustrating the cortical areas in fishes and amphibians.

FREDERICK T. LEWIS, *Harvard Medical School*. (a) The first lymph glands in rabbit and human embryos. Specimens and models illustrating the relation of the atrioventricular valves to the interventricular foramen.

STEWART PATON, *Princeton, New Jersey*. Preparations showing neurofibrillation in relation to the first movements of the vertebrate embryo.

WILLIAM S. MILLER, *University of Wisconsin*. Reconstruction models showing the arrangement of the cartilages in the trachea and bronchi of the Guinea pig. (b) Arrangement of the muscle in the trachea and at the carina tracheæ in various animals.

S. WALTER RANSON, *Northwestern University Medical School*. Sections of the human sciatic nerve showing non-medullated nerve fibers.

FLORENCE R. SABIN, *Johns Hopkins University*. Specimens showing the development of the structural unit in the embryo pig's spleen.

J. PARSONS SCHAEFFER, *Cornell University Medical School, (Ithaca, New York)*. Models showing the development of the lateral wall of the nasal cavity in man.

HAROLD D. SENIOR, *College of Medicine, Syracuse University*. A method of obtaining orientation points in serial sections, for use in plastic reconstructions.

CHARLES F. SILVESTER, *Princeton University*. Preparations showing the presence of permanent lymphatico-venous communications at the renal level in the South American monkey.

GEORGE L. STREETER, *University of Michigan*. Demonstrating for (a) F. H. Busby. Models showing the topography of the cerebral cortex of the opossum.

(b) J. H. STOKES. Two models, showing the facial, vestibular and cochlear nerves with their central connections in the opossum.

(c) H. A. CALHOUN. Models of the medulla oblongata of the opossum.

(d) H. W. STILES. Model showing the Ventricular system of the brain of the opossum.

(e) H. N. T. NICHOLS. Double spinal ganglia.

JOHN L. BREMER, *Harvard Medical School*. Demonstration of unit room No. 203, showing equipment and material used in the first year's course in Embryology and Histology in the Harvard Medical School.

Members of the staff demonstrated models illustrating vertebrate development; made by students in the Harvard Laboratory of Comparative Anatomy.

WEDNESDAY, DECEMBER 29, 9.30 A. M. TO 1 P. M. SESSION FOR THE READING OF PAPERS, FIRST VICE-PRESIDENT WILLIAM S. MILLER AND PRESIDENT JAMES PLAYFAIR McMURRICH, PRESIDING.

ELEXIOUS T. BELL, *University of Missouri*. On the staining of fat in muscle fibers.  
VICTOR E. EMMEL, *Washington University Medical School*. Observations on the differentiation of regenerating epidermal and striated muscle tissue in the lobster.

ARTHUR E. HERTZLER, *Kansas City, Missouri*. The formation of fibrous tissue.  
GEORGE S. HUNTINGTON, *Columbia University (New York City)*. The development of the thoracic ducts in embryo of the cat (with lantern slides).

EDWIN G. CONKLIN, *Princeton University*. Cell size and nuclear size.

JEREMIAH G. FERGUSON, *Cornell University Medical School (New York City)*.  
1. The hypobranchial arterial system in the Selachiae. 2. The thyroid gland of elasmobranchs, with special reference to its Vascular supply.

CLARENCE M. JACKSON, *University of Missouri*. Electric heating for laboratory apparatus.

The following papers announced on the program were read by title:

CHARLES S. MINOT, *Harvard University*. Notes on an early stage of pregnancy.

HERBERT M. EVANS, *Johns Hopkins University*. Note on the development of the superficial arteries of the head in the human embryo; especially the occipitalis, auricularis posterior and temporalis superficialis.

HARVEY E. JORDAN, *University of Virginia*. A further study of the human umbilical vesicle.

WILLIAM F. MERCER, *Ohio University*. Development of the metacarpal bones in the leg of the sheep.

12 to 1. Address by Professor Doctor Franz Weidenreich of Strassburg, Germany, On the morphology of the blood cells and their relation to each other. (Die Morphologie der Blutzelle und ihre Beziehungen zu einander.)

This address, given at the invitation of Professor Minot and the Executive Committee, was delivered in German. At its conclusion, the Association extended Professor Weidenreich a vote of thanks and appreciation.

WEDNESDAY, DECEMBER 29, 2 TO 4 P. M. DEMONSTRATIONS AS FOLLOWS:

ELEXIOUS T. BELL, *University of Missouri*. Preparation showing fat in muscle fibers.

VICTOR E. EMMEL, *Washington University Medical School*. Preparations showing the differentiation of regenerating epidermal and striated muscle tissue in the lobster.

**JEREMIAH S. FERGUSON**, *Cornell University Medical School (New York City)*.  
(a) Dissection of the hypobranchial system of the dogfish. (b) Sections and total mounts of the thyroid gland of elasmobranchs.

**GEORGE S. HUNTINGTON**, *Columbia University* and **C. F. W. McClure**, *Princeton University*. Models illustrating the development of the jugular lymph sacs in mammalia.

**ARTHUR E. HERTZLER**, *Kansas City Missouri*. Preparations and drawings showing the formation of fibrous tissue.

**PROFESSOR DOCTOR WEIDENREICH**.—A demonstration of a series of preparations showing the morphology of the blood cells and their relation to each other.

### WEDNESDAY, DECEMBER 29, 4 P. M. BUSINESS MEETING.

On motion, the minutes of the Secretary as published in the *Anatomical Record*, Vol. III, No. 1, page 62 to 74, were approved.

The Treasurer made the following report for the year 1909:

Total receipts for the year 1909.....	\$1381.20	
Balance on hand December 24, 1908.....	172.17	
<b>Total.....</b>	<b>\$1553.37</b>	<b>\$1553.37</b>
Expenses of the Secretary, Baltimore meeting.....	\$32.40	
Smoker, Johns Hopkins Club.....	7.60	
Postage and envelopes.....	26.20	
Wistar Institute of Anatomy for 275 subscriptions to American Journal of Anatomy and Anatomical Record at \$4.50.....	1237.50	
Printing.....	19.40	
<b>Total.....</b>	<b>\$1323.10</b>	<b>1323.10</b>
Balance on hand December 23, 1909, deposited in the Farmers and Mechanics Bank, Ann Arbor, Michigan .....		<b>\$230.27</b>

August G. Pohlman reported for the Auditing Committee: "We have examined the accounts of G. Carl Huber, Secretary-Treasurer for the year 1909 and found them correct."

On motion the reports of the Treasurer and of the Auditing Committee were accepted and adopted.

James Playfair McMurrich and Ross G. Harrison, members from this Association of the International Committee on Reformation of Myological nomenclature, reported progress. The committee was continued.

The Committee of this Association, consisting of Charles S. Minot, Franklin P. Mall, James Playfair McMurrich, G. Carl Huber, George A. Piersol, George S. Huntington, in charge of arrangements for the International Congress of Anatomy to be

held in Brussels, August 7 to 11, 1910, through its Chairman, Dr. Minot, reported progress.

The following were recommended by the Executive Committee for election to membership in the Association.

ROBERT P. BIGELOW, Ph.D., Instructor in Biology and Librarian, *Massachusetts Institute of Technology*.  
 DAVID CHEEVER, A.B., M.D., Demonstrator of Anatomy, *Harvard Medical School*.  
 H. K. CORNING, M.D., Professor of Anatomy, *Basel, Switzerland*.  
 VICTOR E. EMMEL, Ph.D., Instructor in Histology and Embryology, *Washington University, St. Louis*.  
 FREDERICK ETHERINGTON, M.D., Professor of Anatomy, *Queen's University, Kingston, Canada*.  
 WILLIAM S. HALSTED, M.D., Professor of Surgery, *Johns Hopkins University*.  
 DAVENPORT HOOKER, M.A., Instructor in Anatomy, *Medical Department, Yale University*.  
 FRANKLIN P. JOHNSTON, A.B., Austin Teaching Fellow, *Harvard Medical School*.  
 J. F. MCCLENDON, Ph.D., Assistant in Histology, *Cornell University Medical School, New York*.  
 MAX MORSE, Ph.D., Instructor in Biology, *College of the City of New York*.  
 ERNEST SACHS, A.B., M.D., Physician and Surgeon, *New York City*.  
 DANIEL M. SHOEMAKER, B.S., M.D., Associate Professor of Anatomy, *St. Louis University*.  
 JAMES M. STOTSENBERG, M.D., Curator and Junior Associate in Anatomy, *Wistar Institute*.  
 FREDERICK TILNEY, A.B., M.D., Associate in Anatomy, *Columbia University, New York City*.  
 LOUIS HILL WEED, A.M., *Johns Hopkins Medical School, Baltimore*.  
 FRANZ WEIDENREICH, M.D., a.o., Professor and Prosector of Anatomy, *Strassburg, Germany*.

On motion, the Secretary was instructed to cast a ballot for election to membership in the American Association of Anatomists of applicants recommended by the Executive Committee. Carried.

The Association then proceeded to the consideration of the constitution placed before this Association at its last meeting by the committee on revision of the constitution, consisting of G. Carl Huber (Chairman), Henry H. Donaldson and Robert R. Bensley, and sent to each member at least one month in advance of this meeting as provided for in Section 2, Article VII, of the constitution.

On motion, the constitution proposed by the committee was considered article for article. Each article was voted on separately and adopted as proposed or as amended. In conclusion the entire constitution was unanimously adopted as a whole, in the following form:



## CONSTITUTION.

## ARTICLE I

SECTION 1. The name of the Society shall be "The American Association of Anatomists".

SEC. 2. The purpose of the Association shall be the advancement of anatomical science.

## ARTICLE II

The officers of the Association shall consist of a President, a Vice-President, and a Secretary, who shall also act as Treasurer. The President and the Vice-President shall be elected for two years, the Secretary for four years. In case of absence of the President and Vice-President, the senior member of the Executive Committee shall preside. The election of all the officers shall be by ballot.

## ARTICLE III

The management of the affairs of the Association shall be delegated to an Executive Committee, consisting of eleven members, including the officers. Two members of the Executive Committee shall be elected annually, and, so far as possible, election of members of the Executive Committee shall be in proportion to the geographical distribution of members. Five shall constitute a quorum of the Executive Committee.

## ARTICLE IV

The Association shall meet at least annually, the time and place to be determined by the Executive Committee. The annual meeting for the election of officers shall be the meeting of convocation week, or in case this is not held, the first meeting after the new year.

## ARTICLE V

SECTION 1. Candidates for membership must be persons engaged in the investigation of anatomical or cognate sciences,

and shall be proposed in writing to the Executive Committee by two members, who shall accompany the recommendations by a list of the candidate's publications, together with references. Their election by the Executive Committee, to be effective, shall be ratified by the Association in open meeting.

SEC. 2. Honorary members may be elected from those who have distinguished themselves in anatomical research. Nominations by the Executive Committee must be unanimous and their proposal with a reason for recommendations shall be presented to the Association at an annual meeting, a three-fourths vote of members present being necessary for an election.

#### ARTICLE VI.

The annual dues shall be five dollars. A member in arrears for dues for two years shall be dropped by the Secretary at the next meeting of the Association, but may be reinstated at the discretion of the Executive Committee on payment of arrears.

#### ARTICLE VII.

SECTION 1. Twenty members shall constitute a quorum for the transaction of business.

SEC. 2. Any change in the constitution of the Association must be presented in writing at one annual meeting in order to receive consideration and be acted upon at the next annual meeting; due notice of the proposed change to be sent to each member at least one month in advance of the meeting at which such action is to be taken.

SEC. 3. The ruling of the Chairman shall be in accordance with "Robert's Rules of Order."

The orders adopted by this Association, which read as follows, were not altered:

Newly elected members must qualify by payment of dues for one year within thirty days after election.

The maximum limit of time for the reading of papers shall be twenty minutes. The Secretary and Treasurer shall be allowed his traveling expenses and the sum of \$10 toward the payment of his hotel bill, at each session of the Association.

That the Association discontinue the separate publication of its proceedings and that the ANATOMICAL RECORD be sent to each member of the Association, on payment of his annual dues, this journal to publish the proceedings of the Association.

Charles S. Minot, as Chairman of the Committee on nominations, placed before the Association the following nominations:

*President*..... GEORGE A. PIERSON.  
*Vice-President*..... CHARLES F. W. McCLURE.  
*Secretary-Treasurer*..... G. CARL HUBER.

*For Members of the Executive Committee.*

IRVING HARDESTY,  
 WARREN H. LEWIS,

ROBERT J. TERRY,  
 FREDERICK T. LEWIS.

On motion, the Secretary was instructed to cast a ballot for the election to the respective offices of the members nominated by the Committee on nominations.

Charles S. Minot moved "That the American Association of Anatomists recommend to the International Congress of Anatomy the appointment of an International Committee to revise embryological nomenclature and prepare a list of standard terms." Seconded and carried.

On motion of Thomas G. Lee, the business meeting was adjourned.

THURSDAY, DECEMBER 30, 9:30 A.M. TO 1 P.M. SESSION FOR THE READING OF PAPERS, SECOND VICE-PRESIDENT, FLORENCE R. SABIN, AND THE PRESIDENT, JAMES PLAYFAIR McMURRICH, PRESIDING. THE FOLLOWING PAPERS WERE PRESENTED:

- J. F. McCLENDON, *Cornell University Medical School (New York City)*. The totipotency of the first two blastomeres of the frog's egg.
- J. PARSONS SCHAEFFER, *Cornell University Medical School (Ithaca)*. Or the genesis of air cells in the nasal conchæ.
- GEORGE S. HUNTINGTON and H. v. W. SCHULTE, *Columbia University (New York City)*. Contribution to the morphology of the mammalian salivary glands.
1. H. v. W. SCHULTE. Development of the salivary glands of the cat.
  2. GEORGE S. HUNTINGTON. Anatomy of the salivary glands in primates. (Only a brief abstract presented.)
- LEO LOEB and WILLIAM F. H. ADDISON, *University of Pennsylvania*. The transplantation of skin of the Guinea pig and the pigeon into other species.
- CHARLES R. STOCKARD, *Cornell University Medical School (New York City)*.
1. The influence of alcohol and other anaesthetics on the developing embryo.
  2. The independent origin and self differentiation of the crystalline lens.
- GEORGE L. STREETER, *University of Michigan*. A new method of dissection of the spinal cord and brachial plexus (Lantern slides)..
- ROBERT J. TERRY, *Washington University Medical School*. The morphology of the pineal region in fishes.
- JOHN WARREN, *Harvard Medical School*. On the paraphysis and pineal region in *lacerta* and *chrysemis marginata*.

- FRANKLIN P. JOHNSTON, *Harvard Medical School*. Development of the glands and villi of the human digestive tract.
- LEONARD W. WILLIAMS, *Harvard Medical School*. The somites of the chick.
- JAMES MURPHY, *Johns Hopkins Medical School*. On the relation of the sulcus lunatus to the visual area in the negro and white brains.
- G. CARL HUBER, *University of Michigan*. (Only brief abstracts presented).
1. On the relation of the notochord to the anlage of the pharyngeal bursa.
  2. A note concerning the caudal end of the notochord in human embryos.
  3. Concerning embryonic remains of the caudal end of the neural canal in the human embryo.

The following papers announced were read by title:

- CHARLES F. SILVESTER, *Princeton University*. On the presence of permanent lymphatic-venous communications at the renal level in the South American monkeys.
- FREDERICK TILNEY, *Columbia University (New York City)*. Comparative histology of the hypophysis.
- CHARLES R. BARDEEN, *University of Wisconsin*. Practical state board examination in anatomy.

Owing to the absence of Dr. Bardeen and at the suggestion of the Executive Committee, the Association voted that Dr. Bardeen's paper be printed in the ANATOMICAL RECORD and that the President appoint a committee to collect data and consider the question of State Board examinations and report to this Association at a future meeting.

The President appointed as such Committee, Charles R. Bardeen (Chairman), Franklin P. Mall, and George A. Piersol.

THURSDAY, DECEMBER 29, 2 TO 5 P.M. DEMONSTRATIONS AS FOLLOWS:

- ROBERT J. TERRY, *Washington University Medical School*. (a) Specimens and drawings illustrating the morphology of the pineal region in teleosts. (b) The velum transversum of Opsanus, a true choroid plexus.
- JOHN WARREN, *Harvard Medical School*. Models showing the paraphysis and pineal region in lacerta and chrysemis marginata.
- CHARLES R. STOCKARD, *Cornell University Medical School (New York City)*. A sagittal section of a 2.2 mm. human embryo with 8 primitive segments.
- H. v. W. SCHULTE, *Columbia University (New York)*. Preparations illustrating the development of the salivary glands in the cat.
- CLARENCE M. JACKSON, *University of Missouri*. Electric heater and thermostat for paraffin ovens.
- LEO LOEB and WILLIAM H. F. ADDISON, *University of Pennsylvania*. Microscopic preparations of the skin of Guinea pig and pigeon after transplantation to other species.
- FRANKLIN P. JOHNSON, *Harvard Medical School*. Models showing the development of glands and villi of the human digestive tract.

- CHARLES A. TODD, *Washington University Medical School*. Specimens illustrating a plan for a human anatomical museum.
- G. CARL HUBER, *University of Michigan*. Preparations showing (a) The relation of the notochord to the anlage of the pharyngeal bursa; (b) The caudal end of the notochord in human embryos; (c) Embryonic remains of the caudal end of the neural canal in human embryos.

G. CARL HUBER, *Secretary-Treasurer,*  
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# NOTE ON THE SULCUS LUNATUS IN NEGRO AND WHITE BRAINS AND ITS RELATION TO THE AREA STRIATA

JAMES B. MURPHY

*From the Anatomical Laboratory of the Johns Hopkins University*

WITH SIXTEEN TEXT FIGURES

The recent critical work of Professor Mall<sup>1</sup> on the evidences of any racial characteristic of the brain that can be made out by our present methods, involving as it does both his own work and an analysis of the evidence in the literature, must make one skeptical of results that are positive in their nature.

Probably no area of the cortex has been subjected to so much study as the occipital cortex, in the hope of some evidence of racial characteristics. In 1904 Elliott Smith<sup>2</sup> suggested a method of study of the occipital lobe which appeals to reason as being a little less crude than the methods of weighing and comparing surface form and folds, and one that yielded Professor Smith results which stimulate a further application of the method. The method in a word is this: the histological picture of the cortex in the calcarine region, namely, the visual area, is sufficiently marked to be distinguished in thin, freehand sections of a fresh brain, or of a brain hardened in formalin. The characteristic of this area is the so-called stripe of Gennari or line of Vicq d'Azyr. This stripe is readily seen with the naked eye and Smith pointed out that its limits are sharp rather than indefinite, so that it is not necessary to stain sections for fibers by the Weigert method in order to mark out the area striata. Having marked out the area striata by means of freehand sections, he studied the sulci of the calcarine area

<sup>1</sup>Mall. On several anatomical characters of the human brain, said to vary according to race and sex, with especial reference to the weight of the frontal lobe. *Amer. Journ. of Anat.*, vol. 9, 1909.

<sup>2</sup>Smith. Morphology of the retro-calcarine region of the cortex cerebri. Studies in morphology of the human brain. No 1. The occipital region. Records of Egypt, Gov. School of Med., vol. 11, 1904. New studies of the folding of the occipital sulci in human brain. *Journ. of Anat. and Physiology*, vol. 41, p. 198, 237.

from the point of view of their relations to the area striata. In the following points, which can be seen in fig. 1, Smith sharpens our definitions of the region (fig. 1-A.) The calcarine fissure, making the well-known stem of the Y, forms the limiting anterior boundary of the area striata in most human brains, which agrees with the findings of both Flechsig<sup>3</sup> and Campbell.<sup>4</sup> Its corresponds to the calcar avis and is primitive in type. Its development corresponds to the differentiation of the cortex of the area (fig. 1-B.) The continuation of the calcarine fissure, namely, the retrocalcarine sulcus, is to be defined as a sulcus within the area striata, not bounding it, and the foldings of the area striata into retrocalcarine sulci is subject to great variations (fig. 1-C). The retrocalcarine sulcus usually extends around the occipital pole from the mesial surface to the lateral surface. This extension has been well-named by Cunningham, the external calcarine sulcus. Likewise, the area striata, surrounding this external calcarine sulcus, extends around the pole of the occipital lobe to the lateral surface of the brain and usually comes into relation to a curved sulcus, which Smith calls the lunatus, the homologue of the Affenspalte.

This study was begun to test the relation of the area striata to the sulcus lunatus, which it will be seen is a reaching out toward comparing the histological structure of different brains. The number of brains studied has been extremely small, but since the work is unavoidably interrupted for a few years, it was deemed best to publish the results that have been obtained.

The sulcus lunatus Professor Smith considers definitely related to the area striata. To quote his words: "In all mammals (with the exception of man in some cases) the stripe of Gennari extends to the lateral aspect of the hemisphere. In apes, and in most cases in man also, the anterior crescentic edge of this area striata pushes itself forward in such a manner that a deep cleft, a simple sulcus or a mere pucker is formed in front of the advancing

<sup>3</sup> Flechsig. Ueber Untersuchungs-methoden der Grosshirnrinde. Berichte math-phys. Klasse d. K. Sachs. Gesellschaft, Leipzig, 1904, and *Arch. f. Anat. u. Phys.*, Anat. Abt. 1905.

<sup>4</sup> Campbell. The Localization of cerebral function. Cambridge University Press.

edges, like a trough in front of a wave. This trough is often bridged by one or several folds separating the deeper parts one from the other . . . . The sulcus lunatus is a depression formed by the forward projection of the cortical area containing the striata of Gennari." It is found in many forms and positions on the surface of the brain.

The material for this work was selected at random from the collection in the anatomical department of the Johns Hopkins University. The drawings were made and the area striata was plotted before the records of the race were looked up, in all but one or two cases in which brains were chosen to even up the series, so as to rule out the personal equation as much as possible. There were however no records kept in the department to show the type of negro, whether full-blooded or mulatto. The drawings are geometrical tracings, made by means of the very accurate projecting apparatus made by Hermann of Zürich. Great care was taken to get the brains in as uniform a position as possible. In plotting the area striata, very thin sections were made with a razor, perpendicular to the surface of the cortex and extending just through the gray matter, after the manner Smith describes. My experience agrees with Professor Smith's in regard to the definiteness with which the area striata can be marked out with the naked eye (see *Journ. of Anat. and Phys.*, vol. 41, p. 240). I have not controlled the findings with Weigert sections. Starting with the anterior calcarine region and working back through the posterior and external calcarine sulci, these sections were taken at regular intervals, plotted and replaced in order to keep the brain intact. Except in a few poorly preserved brains, the stripe of Gennari showed up so distinctly that there was little trouble in making it out.

The type of the drawings made is shown in fig. 1. The mesial surface of the two slides was carefully drawn by the projection apparatus, then the lobes fitted together, and a third tracing of the sulci was made, looking directly down on the occipital pole. The circle in fig. 1-B shows the area used in the other illustrations. The shaded region is that part of the cortex bearing the stripe of Gennari. The mesial surface drawings of the other brains are not shown, as the extent in the two races is practically the same.

In studying the series of ten negro brains (figs. 1 to 10) certain points are readily seen in the drawings. First, as Smith pointed out, there is a great variation both in the extent of the area striata on the lateral surface of the brain and in its relation to the sulcus lunatus. The sulcus lunatus tends to be more definite on the left side than on the right (see figs. 7 and 8). As was noted by Smith, the area may touch the sulcus, but more often does not quite reach it. In the series of ten negro brains, there is a definite lunatus making an anterior boundary for the area striata on the left side in eight cases, and a doubtful one in two (figs. 3 and 9). In fig. 3 the limiting sulcus (X) is slightly farther from the area striata; while in fig. 9 it is very small. On the right side there is a definite lunatus in three brains, figs., 1, 4 and 6, a possible one in four, figs. 2, 5, 9, and 10, while in three, figs. 3, 7 and 8, no definite lunatus is made out. Fig. 10 is from a mulatto.

In the series of six white brains, the area striata on the lateral surface is somewhat less extensive than in the negro series. The presence of a sulcus lunatus is certainly not as marked as in the other series. In one brain (fig. 16), it is very definite on the right side, and is probably present in the left, and it is interesting to note that this is the brain of an undersized man, who from the statements of the hospital history was probably a degenerate. In this case the striated area is much more extensive than in the other white brains. All of the rest must be regarded as having a most indefinite lunatus or none. The unbroken extension of the posterior calcarine sulcus to the lateral surface, though present in several instances at least on one side, is less often noted than in the negro series. There are in several instances crescentic folds in these white brains which might possibly be considered the lunatus. I have not so classified them because they are some distance from the area and bear no constant relation to it and hence, in my judgment, should not be classed as the sulcus lunatus.

Duckworth,<sup>5</sup> in his article on the brains of aboriginal natives of Australia, found that the retrocalcarine sulcus was continued to the lateral aspect of the hemisphere in 37.5 per cent of the cases.

<sup>5</sup> Duckworth. Brains of the aboriginal natives of Australia. *Journ Anat. and Phys.*, January, 1909.

This extension he regards as a simian characteristic. He finds it occurs much more often on the left side than on the right. The simian sulcus, occipitalis lunatus, is more frequently found on this side also. These same facts have been observed in my series of negro brains.

From this limited series it would seem that the sulcus lunatus is often the anterior limit of the visual cortex on the lateral surface of the brain but cannot be regarded as nearly so constant as the relations of the calcarine fissure and retrocalcarine sulcus. In contrasting the two series for racial distinctions, while it is true that the negro series shows a tendency to a more marked lunatus, it cannot be regarded as a racial characteristic since there are negro brains without it, and a white brain with a definite lunatus. This study however, confirms Smith's idea that the above method brings out the variations in the visual area, which, whether racial or individual, it is worth while to investigate.

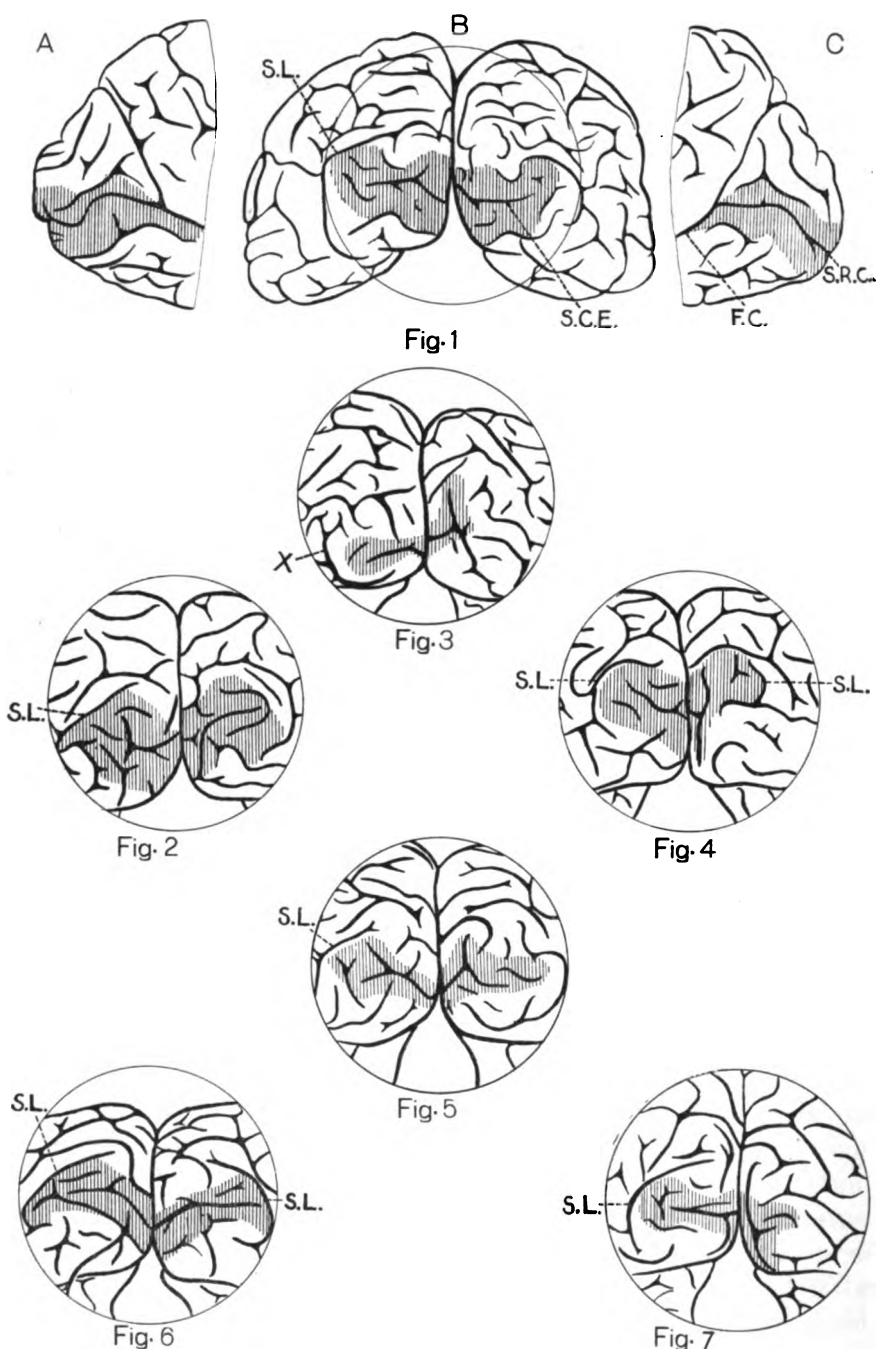


FIG. 1. Tracings of the occipital lobe showing the area striata (shaded) both on the mesial and lateral surfaces. Brain of a negro (Col. No. 3027).

F. C., fissura calcarina; S. R. C. sulcus retrocalcarinus; S. C. E., sulcus calcarinus externus; S. L., sulcus lunatus.

FIGS. 2 to 10. Tracings of the occipital pole of negro brains, showing the area striata on the lateral surface.

S. L. sulcus lunatus; X, a possible sulcus lunatus.



Fig. 9

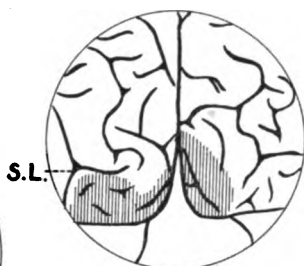


Fig. 8



Fig. 10



Fig. 12



Fig. 11



Fig. 13



Fig. 15

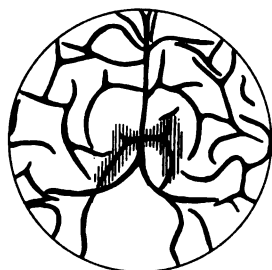


Fig. 14



Fig. 16

FIGS. 11 to 16. Tracings of the occipital pole of white brains, showing the area striata on the lateral surface.

S. L., sulcus lunatus.





# THE HISTOLOGY OF THE NASAL MUCOUS MEMBRANE OF THE PIG<sup>1</sup>

NATHANIEL ALCOCK

*From the Zoölogical Laboratory of Northwestern University*

WITH FIFTEEN FIGURES

With the introduction of the methylene blue and the Golgi methods there began a new era of investigating nervous tissues. When these methods were applied to the histology of the nasal mucous membrane, they made clear for the first time the connection of undoubted olfactory fibers with the sensory cells. Prior to the use of these methods a number of interesting observations on the structure of the nasal epithelium had been made—extending, in fact, over a period of thirty years—but the results obtained have been largely superseded by later investigations.

The pioneer group of researches upon the nasal mucous membrane includes the work of Eckhard ('55), Ecker ('55), Max Schultze ('56, '62), Exner ('72), Cissoff ('74), and von Brunn ('75, '80).

The papers of this period worthy of greatest consideration are those of Max Schultze. In 1856<sup>2</sup> he made the first good analysis of the histology of the nasal mucous membrane, observing all classes of vertebrates and giving sketches of two kinds of cells in the pike, frog, owl and man. His figures were so good that two of them (of frog and man) have been frequently republished.

The two kinds of cells that he designated are (a) six-sided prismatic supporting elements—non-ciliated in the olfactory region, but ciliated in the respiratory region, and (b) true olfactory cells with a large body, a slender peripheral process, and very fine central process showing varicosities. He was the first to claim that the olfactory cells were the only percipient elements of the sense of smell. He supposed their central

<sup>1</sup> Contribution from the Zoölogical Laboratory of Northwestern University under the direction of William A. Loey.

<sup>2</sup> *Monatsber. d. K. Preuss. Akad. d. Wiss. zu Berlin*, November, 1856. Pp. 504-514.

fibers were the true olfactory fibers but was unable to trace them to the olfactory bulb. He found olfactory hairs on the sensory cells of the frog but not on those of mammals. He also saw stellate cells which he likened to the ganglionic cells of the retina. His paper of 1862<sup>3</sup> of ninety-four pages and five lithographed plates is an extension of his earlier observations and embraces observations on all classes of vertebrates.

It was in 1886 that Ehrlich published his paper on the methylene blue method and described its reaction toward several types of nerve-cells, among them the olfactory sensory-cells. He described these briefly, paying special attention to the central processes of the cells which he observed passing downwards between the central processes of the supporting cells into the submucosa and there being continued as the small fibers of the olfactory nerve. This was an ocular demonstration of the connection that Max Schultze had assumed to exist. The following year Arnstein, employing the same method, published a paper in which he described the connection of the olfactory cells with the olfactory nerve and thus agreed with Ehrlich.

In 1887,<sup>4</sup> Dogiel published the results of his work on the olfactory organ of ganoids, teleosts, and amphibians. His observations indicate a polymorphism of sensory cells of the olfactory membrane. He described three types which have been seen by several other observers (Morrill, '98; Jagodowski, '01).

The observations of Grassi and Castronovo<sup>5</sup> in 1889 by the application of the Golgi method to the olfactory membrane of the dog, strengthened the conclusion that the fibers of the olfactory nerve are connected directly with the nerve-cells of the olfactory membrane. They described the true olfactory cell and also a fine varicose central process descending in a curved course to the subepithelium and there uniting with similar fibers to form the olfactory nerve. They also mentioned free nerve endings in the nasal mucous membrane. They observed no anastomosis of nerve processes although they did show in their figures and described in their text one case where two sensory cells were connected by one fiber. They held that the supporting cells were not connected with the nerve fibers. They described the histology of the three areas of the olfactory membrane, giving particular attention to the boundary or intermediate zone, concluding that in the adult it exhibited the embryonic characteristics of the true olfactory region.

<sup>3</sup> *Arch. f. Mik. Anat.* Bd. 27, 1886.

<sup>4</sup> *Abhandl. d. Naturf. Gesellsch. zu Halle.* Bd. 7.

<sup>5</sup> *Arch. f. Mik. Anat.* Bd. 34, 1889. Pp. 385-390.

It will be noted that up to this time investigators had generally supposed that the direction of growth of olfactory nerve fibers was outward from the brain to the olfactory membrane. In the year 1889 this conception was materially changed through the work of William His on the development of nerve fibers and also through that of Ramon y Cajal. The latter traced fibers of the olfactory nerve to the bulbus olfactorius and there found them terminating in brush-like ends that commingle with the dendrites of the mitral cells and form the olfactory glomeruli. He concluded therefore that the sensory cells in the olfactory membrane are the cell bodies from which fibers of the olfactory nerve arise, and that the fibers of this nerve grow not from the brain to the membrane, but the reverse.

The occurrence of free nerve endings in the olfactory membrane was indicated by Cajal. He gives a sketch showing free nerve endings between the cells of the olfactory membrane. Von Lenhossék observed similar appearances and concluded that they were terminations of the trigeminal nerve.

The work of Van Gehuchten ('90) on the rabbit and that of Retzius ('92) on embryos of the mouse, cat, dog and rabbit and his further paper in 1894 on the fishes confirmed the work of previous investigators, adding certain details that do not require mention here.

Von Brunn's paper of 1892<sup>6</sup> dealing with the nasal membrane of the human body is of especial interest. This observer had published two previous papers on the anatomy of the olfactory apparatus, one in 1875 (Dog, Cat, Rabbit, Sheep, Calf, Fish, Salamander) and another, in 1880 (Rabbit) on the same organ in the rabbit. The third paper (1892) dealt with the olfactory apparatus of man. He gives a complete analysis of the membrane, describing its extent, the different cells found, the limiting membrane (described in the three papers, but first mentioned by Sidky), the nasal cells and the connection of the nerve and the sensory element. On the peripheral ends of the olfactory cells he observed from six to eight short pointed hairs but was unable to say whether or not these were normal structures or artefacts. However, he thought them to be normal structures. He concluded also that the nerve fibers are in connection with the sensory-cells only, and not with the supporting elements.

Jagodowski<sup>7</sup> in 1901, published observations on the olfactory organ

<sup>6</sup> *Arch. f. Mik. Anat.* Bd. 29, 1887.

<sup>7</sup> *Anat. Anz.* Bd. 19, 1901.

of the pike. He described three types of sensory elements corresponding to those mentioned by Dogiel. The types are (a) cylindrical *Riechzellen* of Max Schultze with slender peripheral processes, (b) *Riechstäbchen*, with peripheral processes as thick as the cell-body, and (c) *Riechzapfen*, short cells, near the outer border of the mucosa and lacking a peripheral process. This polymorphic condition of olfactory cells has been observed by Morrill ('98, Selachians) and Grassi and Castronovo have pictured a cell like the *Zapfen* in the olfactory membrane of the dog. He also described a new structure, the smell whips. According to Jagodowski each type of olfactory cell bears on the peripheral end a long slender whip-like process which extends into the nasal cavity. He also saw free nerve endings.

Ballowitz<sup>8</sup>, in 1904, published observations on *Petromyzon fluviatilis*, which corroborated the work of Retzius (1880), also on *petromyzon*. He found but one kind of sensory elements and no transitional forms. According to his observations the olfactory cells are of one type but vary in length according to the position of their nuclei in the membrane. On their peripheral ends he saw 10 to 12 fine pointed hairs which are beautifully represented in his sketches. He observed cilia on the supporting cells.

Work on the development of the nervous elements of the olfactory membrane, such as that of Disse<sup>9</sup> ('96) and Bedford<sup>10</sup> ('04), shows conclusively that the neuroblasts of the olfactory nerve fibers are located within the membrane, and that the nerve fibers grow from the nerve cells toward the brain.

Miss Read's<sup>11</sup> "Contribution to the Knowledge of the Olfactory Apparatus in Dog, Cat and Man" ('08), embraces the study of the gross anatomy of the olfactory nerve, as well as a description of the histological structure of the olfactory epithelium illustrated by many figures. Her work also includes a study of the organon vomero-nasale. Her sketches of the sensory-cells of the cat, stained with methylene blue, show the character of these cells very well. She found free nerve terminations in the olfactory membrane, which she regards as derivatives of the trigeminal nerve. She determined that there is no anastomosis of the nerve fibers, but that they preserve their individuality from the nasal epithelium until they terminate in the glomeruli of the

<sup>8</sup> *Arch. f. Mik. Anat.* Bd.64, 1904.

<sup>9</sup> Marburg. *Sitzungsbr.*, October, 1896.

<sup>10</sup> *Jour. Comp. Neurol. and Psychol.*, vol. xiv, 1904.

<sup>11</sup> *Am. Jour. Anat.* May, 1908.

olfactory bulb. She finds in the epithelium of the organon vomeronasale cells apparently identical with the sensory-cells of the olfactory mucosa.

More extended reviews of literature are found in Disse (*Ergeb. d. Anat. u. Entwickl.*, Bd. 11, 1901), Jagodowski (*Anat. Anz.*, 1901), and Miss Read (*Am. Jour. Anat.*, May, 1908). The last mentioned paper was not available until I had completed my work.

#### OBSERVATIONS

The observations, the results of which follow, were undertaken with a view to supplying a purely objective account of the form of the various cellular elements entering into the composition of the nasal mucous membrane, and to determine their normal arrangement. The work was begun in September, 1906, in the Zoölogical Laboratory of Northwestern University, and was carried on for two years. From the beginning it has been under the direction of Prof. William A. Locy, whom I wish to thank for assistance in the observations and preparation of the manuscript. Observations were begun on the rabbit, but by comparison the nasal mucous membrane of the pig was found to give better pictures of the cellular elements, and the present account is confined to descriptions of pig material. I also had for comparison a large series of sections of the nasal-mucous membrane of the rabbit made by Miss Caroline Jaycox.

The pig material used consisted of embryos from six to twelve inches long obtained from the uterus of the parent about thirty minutes after the latter had been killed. In all cases the young specimens were found alive and in good condition. After removal from the uterus the head of the embryo was severed from the body, and split into halves. The nasal cavity was opened by cutting away the septum, and either the whole placed in the fixing or macerating fluid or the turbinal bones removed and placed in the desired liquid.

Two general methods have been followed: That of maceration and that of the study of sections.

Several of the standard macerating fluids were employed. It was found, however, that 25 per cent to 30 per cent alcohol gave

good results with this material. The pieces of tissue were placed in the liquid for a period of thirty-six to forty-eight hours and in a few cases good results were obtained from material which had remained in the alcohol from seventy-two to ninety-six hours. The length of time necessary varies with the age of the pig. The pieces were then transferred whole to a solution of equal parts of 50 per cent alcohol and glycerine in which they were first teased with needles and then shaken vigorously in small bottles. In the majority of cases the cells were found floating free and in clusters in the liquid. To the solution containing the isolated cells were added a few drops of picro-carmin which, in a few hours, stained the cells very satisfactorily. A few drops of this material was then mounted and the coverslip sealed with glycerine jelly and gold-size cement.

For fixing fluids Van Gehuchten's, Müller's, 5 per cent formalin, formalin-acetic, corrosive-acetic, picro-sulphuric, and Zenker's were used. The best results came from the fixation by the Zenker solution. For relatively thick sections the pieces of material were imbedded in celloidin and for thinner sections paraffin was used. The most common and best stains used were iron alum and hæmatoxylin, and eosin and Delafield's hæmatoxylin. The sections range from three to eight microns in thickness.

The larger celloidin sections, after imbedding, were decalcified in 5 per cent nitric acid for twenty-four hours.

In the pig there are five chief ethmo turbinal folds each of which is lobulated. The arrangement exhibited in Fig. 1, which is a drawing of a section cut in a plane perpendicular to the long axis of these folds of a pig embryo twelve inches long, is as follows: The five main folds (F 1, 2, 3, 4, 5) each have a cartilage supporting tissue covered with a relatively thick epithelium. Each fold, with the exception of the second, extends to the side wall of the cavity. The lateral surfaces of the several main folds are elevated into ridges which run horizontally. The first main fold shows 12 of these ridges. The second, which seems to be a division of the first, 8; the third, 11; the fourth, 7; the fifth, 4. Between the second and third main folds are two smaller ones (F 6 and 7); between the third and fourth and between the fourth and fifth are also seen smaller folds (F 8, 9).

In fresh material of the stages examined the epithelium of the nasal cavity is apparently of uniform color. It was not possible to distinguish areas of a yellowish tint indicating the distribution of the olfactory cells, as has been observed in many vertebrates.

The epithelium in fresh condition is so tender that it is almost impossible to remove it from the cartilage upon which it lies, but after fixation with Zenker's fluid this can easily be done even from the turbinal folds. However, it was found that the epithelium of these folds could best be sectioned by leaving it attached to the cartilage and dividing the entire piece into six to eight blocks.

As there is no easily discernible color variation in the different parts of the nasal mucous membrane of pigs of this age, the different areas can be distinguished only by a study of their finer anatomy. On the basis of the thickness of the epithelium and of its component elements two regions can easily be distinguished. One, designated by the earlier investigators as the respiratory region, includes the epithelium of the outer side, the upper, and the lower walls of the nasal cavity and certain parts of the turbinal folds and of the septum. The second, the olfactory region, embraces the sensory epithelium found on the turbinal folds and parts of the septum. My study of its structure was limited to that part on the ethmo-turbinal folds. A third area, the intermediate zone, described at length by Grassi and Castronovo in the dog, can be distinguished in the pig.

*Respiratory Region.* The epithelium of the respiratory region (Fig. 2) is from .037 to .041 mm. in thickness. It is composed of two kinds of cells, cylindrical epithelial cells (*Sup. C.*) and the basal cells (*Bas. C.*). The cylindrical cells (see also Fig. 3) extend across the epithelium from the free surface to the basement membrane. Their large oval nuclei lie a little above the middle horizontal plane of the section. The majority of them show a large oval nucleolus. The peripheral process of each cell extends to the surface where it expands slightly into a dome-shaped disc above the limiting membrane. On this disc are found fifteen to twenty-five slender hairs (*Cil.*) which average about .0065 mm. in length. The central process (*Cen. Pr.*) of each cylindrical



cell extends from the region of the nucleus almost to a pointed end which rests on the basement membrane. These cells show no branching or forking. In the spaces between the pointed ends are the basal cells (*Bas. C.*) which are conical in shape with their large bases turned toward the basement membrane. The nuclei of the latter are spherical.

The sub-mucosa of this respiratory region is from two to three times the thickness of the epithelium itself. It is made up of loose elastic connective tissue and in it are to be seen numerous blood vessels and many of Bowman's glands.

*Olfactory Region. (a) The Supporting Elements.* The olfactory membrane, containing the sensory nervous elements, is confined to the epithelium of the turbinal folds. It has been found on all five of the folds, both on the surface exposed to the nasal cavity and on the lateral surfaces as well. The sensory olfactory membrane varies in thickness from .062 to .07 mm. It is composed of three types of cellular elements, namely, two kinds of supporting elements and the sensory cells. The supporting elements are the cylindrical cells (Figs. 4, 10, 11, 12) and the basal cells (Fig. 12, *Bas. C.*) The latter are similar to those found in the respiratory region. The cylindrical cells are different from those of the respiratory area. Their nuclei which, like those already described, are large and oval, lie in a plane near the limiting membrane, relatively higher in position than those of the supporting cells of the respiratory region. These nuclei stain very deeply with iron hæmatoxylin and also with hæmatoxylin and eosin (Figs. 10, and 11, *Sup. C.*) The peripheral portion of these cells extends to the limiting membrane in a thick process nearly as wide as the cell in the region of the nucleus. The supporting cells in the olfactory region of the pig do not possess cilia. The central process is fine, frequently branched, or forked (Fig. 4), and extends to the basement membrane. Between the forks of the supporting cells are the basal cells in the same relative position as they are in the respiratory area. In no case, neither in the sectioned material nor in the macerated preparations, has a nerve fiber been seen in connection with a supporting cell.

*Olfactory Region. (b) The Sensory Cells.* The sensory elements

of this region, isolated by maceration, are shown in Figs. 5, 6, 7, 8, 9, and in normal position in the membrane in Figs. 10, 11, and 12. As these figures show, the olfactory cells are bi-polar elements, the large oval cell body, which contains a nucleus of nearly the same shape, giving rise to a central and a peripheral process. The olfactory cell bodies lie at varying levels in the epithelium below the plane of the nuclei of the supporting cells. In the lower half of the cell body is the large ellipsoidal nucleus which, with iron alum and hæmatoxylin, does not stain quite so deeply as the nuclei of the supporting cells. The peripheral process arises from the tip of a conical mass of cytoplasm which makes up the upper half of the cell body. This process varies in length in different cells, depending of course upon the position of the cell body within the membrane. The diameter of the process varies from one-fifth to one-third that of the cell body, and extends to the surface and passes through the limiting membrane enlarging in this region into a knob (*Kn.*) whose diameter is one and one-half to two times that of the process itself. On this knob are to be seen four to eight hair-like processes, extending above the knob into the cavity of the nose. In both the macerated material and in the sections the olfactory hairs vary in length from .008 to .001 mm., or a trifle longer than the cilia of the supporting cells of the respiratory region. Unlike the latter they are thick and instead of being pointed are not only blunt but in some cases they seem to have more or less thickened ends. In the sections stained with iron alum and hæmatoxylin and in the macerated material stained with picro-carmin, the bases of these cilia, which are equatorially arranged around the knob of the process, are stained very deeply and look like small dots. It is possible to see olfactory hairs in all sections where the knobs are shown, but in the macerated material they do not occur so frequently and in a great many cases are broken off. Where these hairs do not show, however, the small dots representing the bases of them, can easily be seen. This absence of olfactory hairs from so many of the sensory elements in macerated material may be explained by the treatment which the cells received in being separated. I conclude that the olfactory hairs are normal structures and not artefacts.

At different levels along the peripheral processes of the olfactory cells occur slight enlargements (Figs. 6, 7, 8). These show best in the isolated cells. As many as two have been seen on the same cell. Similar enlargements are shown in the figures of Max Schultze (1856, 1862) and in those of Von Brunn (1892).

Below the nucleus of the sensory cell the cytoplasm is drawn directly to a point which in the majority of the cells is in a line with the long central axis of the cell body, but sometimes it is found to one side of it. From this point emerges a long, slender, varicose fiber, (*Cen. Pr.*), which extends downward in a curved or wavy course toward the sub-mucosa passing between the feet of the supporting cells. It passes through the basement membrane into the sub-mucosa and there is lost in the connective tissue. In the sub-mucosa several of these fine fibers come together to form the smaller branches of the olfactory nerve, but the fibers do not anastomose. In the macerated preparations the cells possess only a very short fiber, and often none at all. By maceration, the central processes, like the olfactory hairs, are easily broken off.

In the sectioned material as well as in the macerated preparations have been seen oval cell bodies, possessing varicose nerve fibers like those of the sensory elements just described, but having no peripheral processes (Figs. 10, 11, 12). In the case of the sections it is plain that this appearance is due to the fact that the plane of the section does not pass through the peripheral process but only through the cell body. In Fig. 15A is shown the cell body of such an element and in Fig. 15B is shown the same region of the next section to it and in it is seen the peripheral process of the sensory cell (*Per. Pr.*), whose body in Fig. 15A might be mistaken for a uni-polar element. Many similar cases have been examined and in all but a very few it was possible to find in the neighboring sections the missing peripheral processes. In like cases in the macerated material it could sometimes be seen that the process had been broken off in the maceration. There is not enough evidence from my observations to justify the separation of the sensory cells into the two classes of uni-polar and bi-polar elements. It appears that they are all bi-polar.

The sub-mucosa of this region resembles that of the respiratory area except that it is much thicker and contains a greater number of Bowman's glands.

Fig. 9 shows the peripheral end of an olfactory cell broken away from its cell body and bearing eight olfactory hairs.

Figs. 10, 11 and 12 represent camera drawings of sections showing the various histological elements of the mucosa in their natural position. It is to be noted that the knob-like endings of the peripheral processes are situated in front of the limiting membrane. They are large and the olfactory hairs are long.

Fig. 13 shows a sensory cell in section with its nucleus near the basement membrane, and, consequently, a very long peripheral process.

The results obtained indicate the success of Zenker's fluid followed by iron hæmatoxylin staining. An examination of the published drawings of the histology of the nasal membrane will show that it is infrequent to find the knob-like eminences and the olfactory hairs so well exhibited as in the pig material upon which the above observations are based. The sketches that show the hairs best developed are those of Ballowitz<sup>12</sup> in Petromyzon.

*The Intermediate Zone.* This zone lies between the two regions just described and exhibits the characteristics of each. It is the shading of the one into the other. It is thinner than the olfactory region, and thicker than the respiratory. Besides the sensory elements it possesses supporting cells of three types. Of the latter the basal cells are similar to those of the other two regions. The long supporting cells proper can be divided into two classes on the basis of possessing or lacking cilia. Those that do bear cilia (Fig. 14, *Sup. C.*)—are very similar to the supporting elements of the respiratory area. They extend across the full width of the membrane and their central processes are pointed and rest upon the basement membrane. The other supporting elements lack cilia, and in this regard are like those of the olfactory region, but their central processes do not appear to be forked.

The sensory elements of this region are not as long as those

<sup>12</sup> *Archiv. f. Mik. Anat.* Bd. 64, 1904.

of the olfactory area, and are fewer in number. In the well differentiated olfactory region about 70 per cent of the cells appear to be nerve elements, while in the middle of the intermediate zone about the same percentage of the elements are supporting cells. The nerve cells of this zone become more and more scattered as the respiratory region is approached.

A differential count of the isolated cells from the entire turbinal membrane shows that 49 per cent are olfactory elements.

**SUMMARY.** The facts of observation briefly summarized are as follows:

1. The nasal mucous membrane of the pig is divided into three areas: The olfactory, characterized by the predominance of sensory cells; the respiratory region, characterized by the absence of sensory cells and the presence of ciliated supporting cells; and, third, the intermediate zone, characterized by the presence of a small number of sensory cells and both ciliated and unciliated supporting cells.

2. There are supporting cells of three easily distinguishable types, viz: the unciliated of the olfactory region; the ciliated of the respiratory region; and the basal cells common to the three regions. Besides these there are the ciliated cells of the intermediate region which differ slightly from those of the respiratory area, and also, the unciliated supporting elements of the intermediate zone which are not identical with those of the olfactory region.

3. The sensory cells so far as observed are of one type, bi-polar. The peripheral process is slender and on its free end is a knob bearing five to eight hair-like processes. The nerve fiber passing centrally, is fine and beset with varicosities. The fibers unite to make up the bundles of the olfactory nerve.

4. No connection has been observed between nerve fibers and the supporting elements of any area.

## EXPLANATION OF FIGURES

FIG. 1. Section of the ethmo-turbinal folds of a pig embryo 12 inches long. The section is cut in a plane perpendicular to the long axis of the folds.

FIG. 2. Section through the respiratory region of the second turbinal fold of a pig 11 in. long. *Bas. C.*, basal cell; *Cil.*, Cilia; *Cen. Pr.*, central process; *Nu.*, nucleus; *Sup. C.*, supporting cell.

FIG. 3. Isolated supporting cell of the respiratory region macerated in 25 per cent alcohol.

FIG. 4. Single supporting cell from the olfactory region of the pig. *L. M.*, limiting membrane; *Nu.*, nucleus; *Cen. Pr.*, central process.

FIGS. 5, 6, 7, 8. Sensory cells from the olfactory membrane of the pig drawn to the same scale. Showing variations in the length of the peripheral process. *Cen. Pr.*, central process or nerve fiber; *Kn.*, olfactory knob; *Nu.*, nucleus; *Olf. H.*, olfactory hair, *Per. Pr.*, peripheral process.

FIG. 9. Peripheral end of an olfactory cell bearing eight olfactory hairs.

FIG. 10. Section through the olfactory membrane of a pig embryo  $6\frac{1}{2}$  in. long. From the second ethmo-turbinal fold. *Olf. H.*, olfactory hairs; *Kn.*, terminal knob of olfactory cell; *Olf. C.*, olfactory cell; *Nu.*, nucleus; *Cen. Pr.*, central process or nerve fiber; *Bas. C.*, basal cell; *Bas. Mem.*, basement membrane.

FIG. 11. Olfactory membrane of the lateral surface of the first ethmo-turbinal fold of a pig embryo  $6\frac{1}{2}$  in. long. Reference letters same as in Fig. 10.

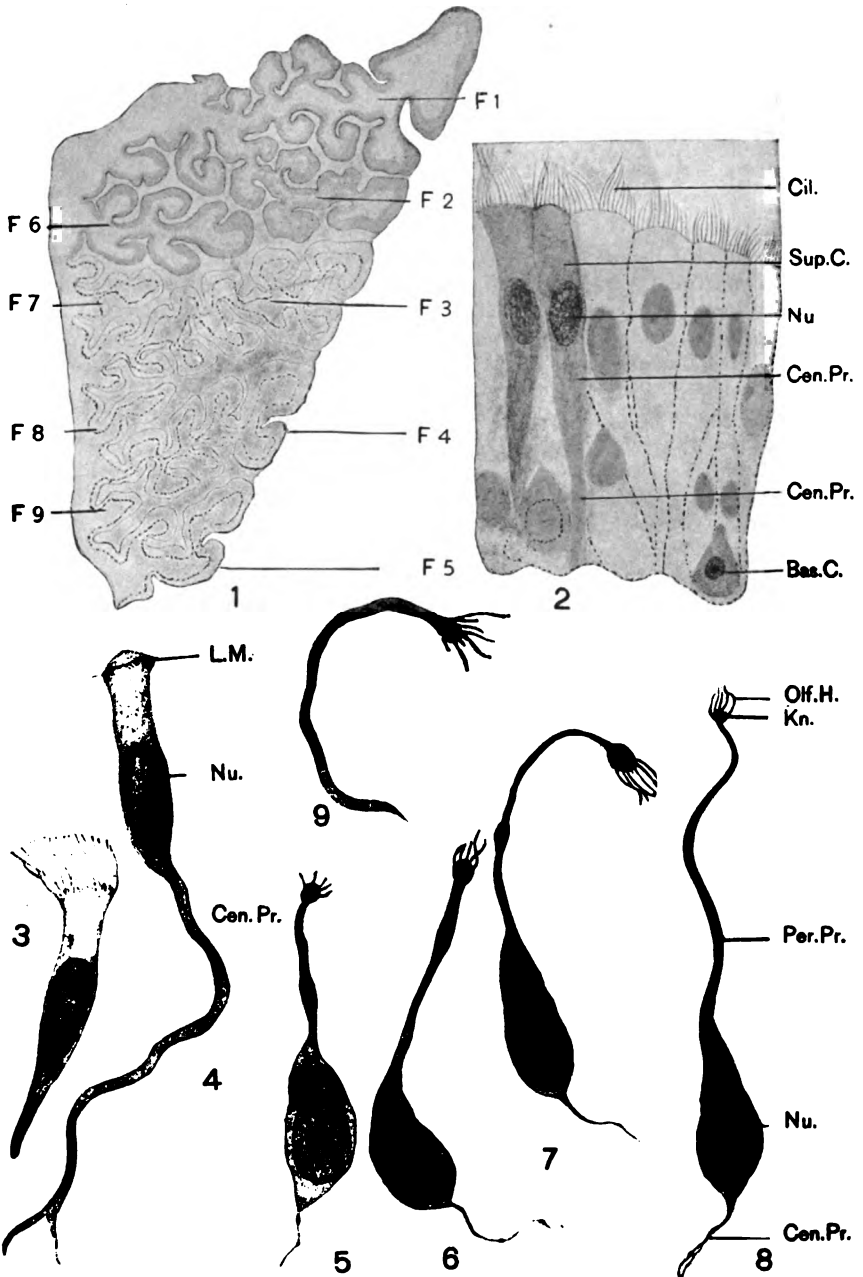
FIG. 12. Olfactory membrane from the same specimen and the same fold as in Fig. 11; *Lim. Mem.*, Limiting membrane.

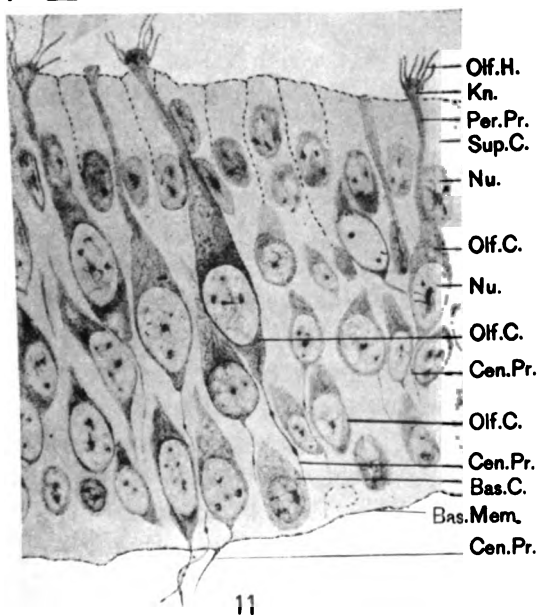
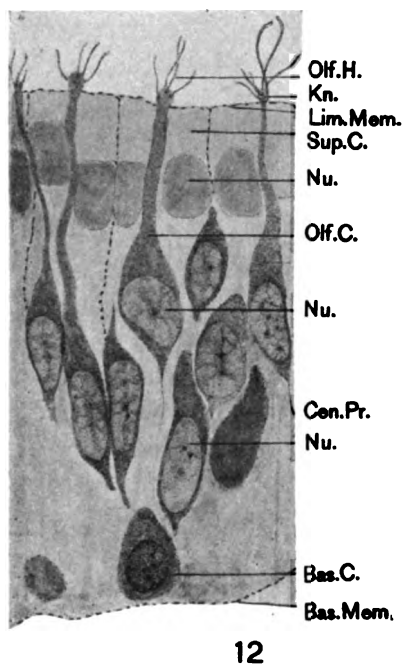
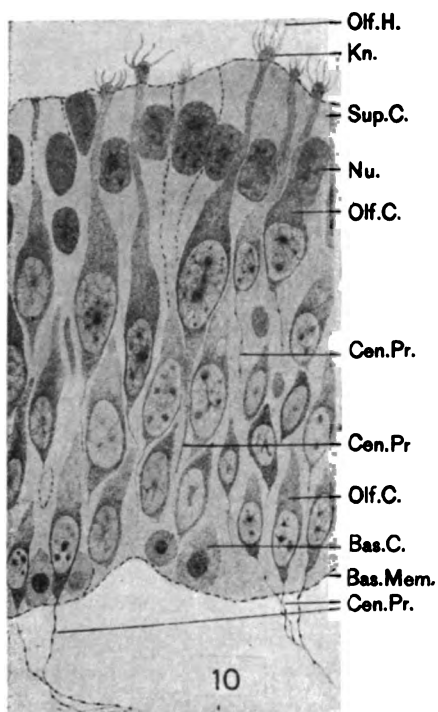
FIG. 13. Section through the olfactory membrane of a pig embryo  $6\frac{1}{2}$  in. long, showing one olfactory cell with a very long peripheral process.

FIG. 14. Section of the olfactory membrane in the intermediate zone from the first ethmo-turbinal fold of a pig embryo  $6\frac{1}{2}$  in. long.

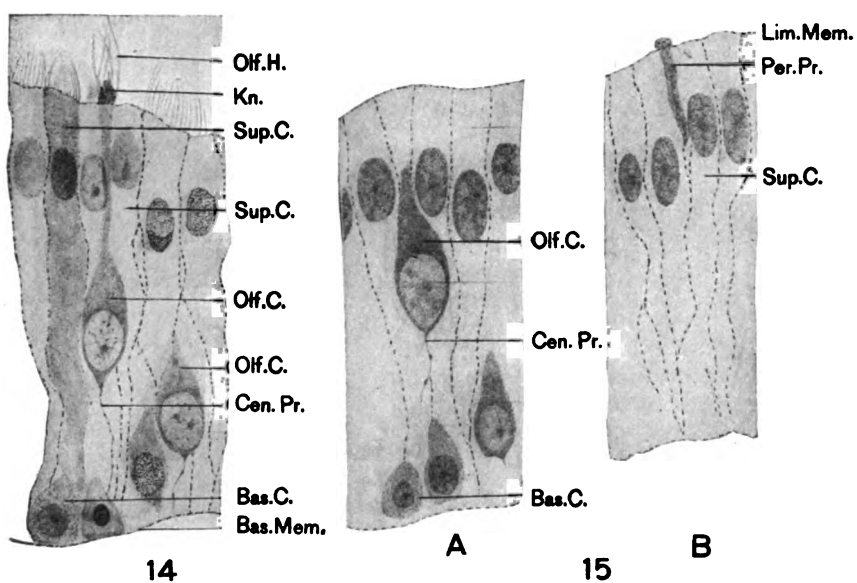
FIG. 15 A. Section through the intermediate zone of the olfactory membrane from the first ethmo-turbinal fold of a pig embryo  $6\frac{1}{2}$  in. long.

FIG. 15 B. Adjacent section to that shown in A. Shows the peripheral process of the chief olfactory cell the body of which is shown in the previous section.









## A SIMPLE ELECTRIC HEATER AND THERMO-REGULATOR FOR PARAFFIN OVENS, INCUBATORS, ETC.

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It is evident that for laboratory apparatus electric heating is in most respects far superior to gas heating, on account of its greater convenience and safety. The latter consideration, that of safety, applies with special importance to those appliances which require continuous heating, and which are left for considerable periods of time without supervision. Those of most importance in biological laboratories are incubators, drying ovens, and paraffin ovens. Experience has shown the danger of fires where gas burners are used for this purpose, even when every reasonable precaution is taken.

For some time, therefore, I have been working on the problem of replacing the gas burners with electric heaters. Various electric appliances for this purpose have been put upon the market, but they are as yet used only to a very limited extent. The chief difficulty seems to be on account of the complexity and cost of the devices now available for electric heaters and thermo-regulators. For example, those now on the market for use with paraffin ovens and incubators cost from \$25 to \$100 or more. An electric heater and thermo-regulator which is simple, efficient and cheap should displace the gas burner in every laboratory.

In such an apparatus we have to consider (1) the heating mechanism; and (2) the thermo-regulator.

(1) For heating purposes, I have adopted the incandescent lamps ordinarily used for lighting purposes. When placed under the oven in a case properly insulated, the light is converted entirely into heat, making a very effective heating apparatus. The cost of operation varies from one-half cent to one cent per hour for each 16 c.p. lamp. For a small oven, or for a comparatively low temperature, one such lamp is sufficient. For a par-

affin oven (water jacket) 12 x 10 x 12 inches, I find that three 16 c.p. lamps will maintain a temperature of 55° to 60° C, which is sufficient for paraffin embedding. Incidentally it may be noted that the ordinary copper ovens radiate heat excessively, and are greatly improved by gluing a layer of asbestos paper over the outer surface.

The case I am using is made of hard wood, lined with asbestos board one-half inch thick (fig. 1). The asbestos board may be procured from the H. W. Johns-Manville Company, New York, or from their branch houses in all the large cities.

The principal objection to the use of the ordinary incandescent lamps is that the carbon filaments burn out somewhat more rapidly than usual, on account of the high temperature. This difficulty is easily avoided by using lamps designed for a higher voltage than is used in the circuit for lighting purposes. If 110 volts are used for lighting, lamps of 125 volts may be used for heating. These would not be suitable for lighting, but are almost as effective for heating, and far more durable, being practically indestructible.

Another objection may arise in cases where the electric current is not available continuously. In our laboratories, for example, the current supplied by the University plant is off from midnight to 5 a.m. This might be a fatal defect with incubators for some purposes, but for paraffin embedding it offers no serious disadvantage. Objects are rarely left in the melted paraffin over night, and even when they are, no harm is done by temporary cooling down of the oven. Before 9 o'clock in the morning the oven is re-heated ready for use. In a drying oven likewise the only disadvantage is the loss of a few hours of maximum temperature.

(2) In order to regulate the temperature, the incandescent lamps are placed in two parallel circuits. In the first circuit is one lamp (or more, if required) of sufficient size (c.p.) to heat the oven up to a point *slightly below* the temperature which it is desired to maintain. In the second circuit is another lamp which, together with the first, will heat the oven to a temperature slightly higher than that desired. The second circuit, however, is connected with a thermo-regulator placed inside the oven and adjusted so that

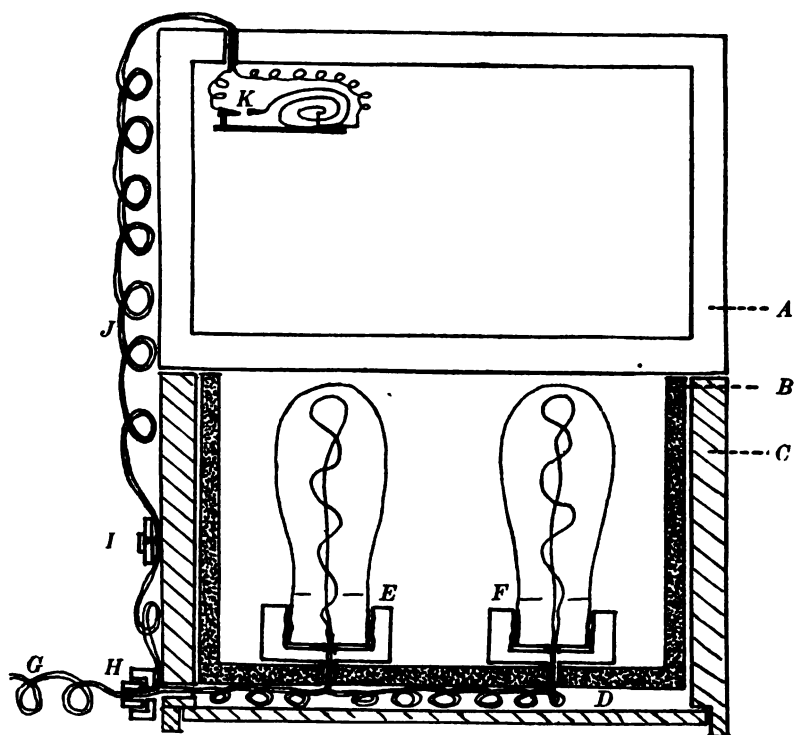


FIG. 1. Diagram of a vertical section showing arrangement of electric heater and thermo-regulator.

A, wall of oven or incubator, resting upon the heater. B, the inner case of the heater, a box made of asbestos board one-half inch thick, and joined together with screws. Cracks and irregularities may be filled with asbestos paste. The outer case, C, is made of oak seven-eighth inch thick. The floor of the outer case is one-half inch thick, and is fastened with screws so as to be easily removable. The inner case rests upon screw-eyes which extend into the walls of the outer case. Between the floors of the cases a space one-half inch deep is left for the wiring. The external measurements of the outer case should correspond in length and width to those of the oven which it supports. The depth of the inner case should be 6 inches, inside measurement, so as to give room for a 16 c.p. lamp, placed upright in a receptacle as indicated in the figure at E and F. For an oven 12 x 10 x 12 inches, to be heated to 60° C., four receptacles should be provided, three to be connected directly in circuit, and one connected by the wiring (J) in series with the thermo-regulator, K, placed inside the oven. A switch, I, should be provided for the latter circuit, and another for the other circuit. G represents the wire, connecting with the source of supply, with attachment plug at H.

when the desired temperature is reached the circuit is broken, being reestablished when the temperature drops slightly.

Various forms of regulators have been invented which can be used for this purpose, all depending upon the varying expansion of substances at different temperatures to make and break the circuit. These regulators may be divided into two classes. In the first, or liquid type, a float rests upon a column of mercury or other liquid. This type is desirable where extreme accuracy of regulation is necessary; but otherwise it is undesirable on account of its cost, complexity, and liability to get out of order. The second class is constructed entirely of solid metal, the contact being made and broken by the varying expansion of a metallic band. While not quite so delicate as the first type, the metallic type is sufficiently accurate for all ordinary purposes. It has the great advantage of simplicity and durability, with nothing to wear out or get out of order. The thermo-regulator which I have adapted for present purposes consists essentially of a coiled steel band, with platinum contact points and is taken from an apparatus used to regulate the heating of rooms. It is manufactured by the Johnson Service Co., of Milwaukee, Wisc., and is quoted by them at \$5, which, however, includes an outer case, thermometer, etc., in addition to the thermostat proper, (which is all that is needed). A number of these thermostats second-hand, but in good condition, can be supplied by Mr. J. A. Whitlow, Heat and Light Station, University of Missouri, Columbia, Mo., at \$2 each. At this rate, the cost of the whole apparatus for heating and regulating an ordinary oven would be less than \$10.

It is important to bear in mind that this thermo-regulator should not be placed in a circuit of more than one-half ampere (one 16 c.p. lamp) on account of burning out the contact points, and also because the weaker the current, the more accurate the regulation. With an 8 c.p. lamp, at ordinary room temperatures, the variation is usually less than  $1^{\circ}\text{C}$ . from the point set.

In case a thermo-regulator of the kind mentioned is not available, one similar in type could readily be constructed. Another form of metallic thermo-regulator has recently been described by Abeken and Cuthbertson (U. S. Naval Medical Bulletin, vol. 4, no. 1, January, 1910).

# THE EVOLUTION OF THE CEREBRAL CORTEX<sup>1</sup>

J. B. JOHNSTON

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WITH TWENTY FIGURES

In the course of a general treatment of the nervous system of vertebrates, published in 1906, the writer stated briefly the results of personal investigations upon a number of topics. In some cases, it has since appeared, the brief treatment did not present adequately the evidence upon which the conclusions were based. Circumstances have greatly delayed the publication of this evidence in more complete form. Recent papers have treated with some fullness the mesencephalic root of the trigeminus, the origin of taste buds and the question of the boundary between diencephalon and telencephalon. The subject of the present paper is to be taken up in a series of short papers with the purpose of demonstrating the early stages in the phylogenetic history of the cerebral cortex. It is but just to say that the great bulk of the evidence now presented upon these various subjects was in hand before the book referred to was written.

The boundary between diencephalon and telencephalon is marked by the velum transversum above and by the caudal surface of the chiasma-ridge below. The telencephalon consists of a ventral portion occupied by the optic chiasma and other decussating fibers and a dorsal portion comprising the corpus striatum, rhinencephalon, cortex, lamina terminalis, tela chorioidea, etc.

<sup>1</sup> The subject matter of this paper was presented at a joint meeting of the Chicago Neurological Society and the Biological Society of the University of Chicago, December 21, 1909, and to the American Association of Anatomists in Boston, 1909.

Neurological studies from the Institute of Anatomy, University of Minnesota, No. 12.

The ventral portion never enters into the evaginations by which the lateral lobes or "hemispheres" are formed. Of the dorsal portion only a part is evaginated in primitive vertebrates and successively more and more of the wall of the unpaired ventricle turns out to become the wall of the lateral ventricle in the higher classes of vertebrates. For reasons of practical convenience in description the distinction between evaginated and non-evaginated parts of the forebrain will be more often used than the more fundamental distinction between dorsal and ventral portions. It is necessary to have unambiguous terms to express this distinction, especially in view of the changes which take place from one class of vertebrates to another, resulting in the transformation or translocation of non-evaginated area into the evaginated. For the non-evaginated wall of the unpaired ventricle in all classes may be used the term *telencephalon medium*. I have suggested (1909) that the term *hemisphere* be used to include all that belongs to one-half of the telencephalon. If, on the contrary, it is desirable to retain for the hemisphere the boundaries given it in the BNA, namely the walls of the lateral ventricles in man, it should have the same significance in lower vertebrates. If this usage is adopted it must be clearly recognized that in various classes of lower vertebrates the term hemisphere will include little or none of the cortical areas which predominate in the hemisphere of man. While the question of this usage is being settled, the term *lateral lobes* used by the older anatomists may be used for the evaginated portion of the forebrain without ambiguity.

In median sagittal section of the embryonic forebrain (figs. 17-20), the roof begins at the preoptic recess and extends around the convex surface to the velum transversum. The median seam is the telencephalic part of the roof plate of His. About the middle of this is seen in many vertebrates a neuroporic recess, marking the point at which the neural tube remained longest in connection with the ectoderm. That part of the roof plate which lies between this point and the chiasma is known as the lamina terminalis; that part which extends from the recessus neuroporicus to the velum transversum is the lamina supraneuroporica. It should be emphasized that *it is only for reasons of practical convenience that the lam-*

*ina terminalis* has been distinguished from the remainder of the roof plate, from which it does not differ in any important way. The neural tube does not end forward in a wide opening whose dorso-ventral diameter is measured by the lamina terminalis after the lateral walls are fused together. Rather, as the neural plate rolls

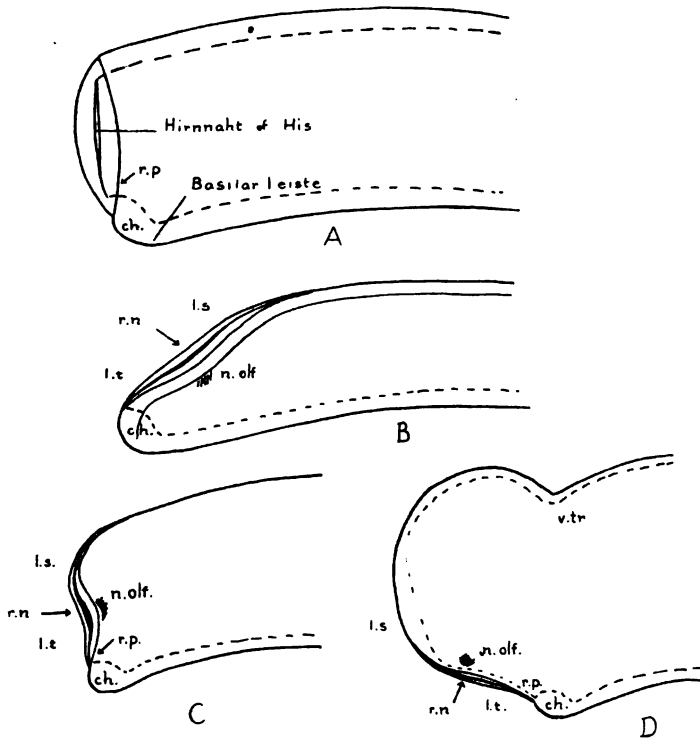


FIG. 1. Schemata to illustrate the two conceptions of the lamina terminalis. In all the sketches the neural tube is viewed from the left and a little in front. In A is represented the common view that the neural tube has a roof plate and a floor plate of equal length. The lamina terminalis is formed by the fusion of the side walls, lettered *Hirnnah of His*. The upper boundary of this would be marked by the neuroporic recess, the lower boundary by the optic chiasma. In B, C and D is illustrated the view stated in the text. The closing of the neural tube is retarded by its relation to the olfactory placode. There is fundamentally a single seam of closure, all of which belongs to the roof plate. The part of this seam between the olfactory nerve and the optic chiasma comes to have the appearance of an endplate (lamina terminalis) because of the forebrain flexure, and this is due to the influence of the olfactory nerve and its centers.



## ABBREVIATIONS

<i>c.a.</i>	commissura anterior	<i>n.olf.</i>	nervus olfactorius
<i>c.call.</i>	corpus callosum	<i>n.term.</i>	nervus terminalis
<i>ch.</i>	optic chiasma	<i>olf.som.</i>	correlation tract between olfactory nuclei and primordium of soma- tic cortex
<i>c.hipp.</i>	commissura hippocampi	<i>par.</i>	paraphysis
<i>c.pall. ant.</i>	commissura pallii ante- rior	<i>p.c.b.</i>	precommissural body
<i>c.post.</i>	commissura posterior	<i>p.h.</i>	primordium hippocampi
<i>c.sup.</i>	commissura superior	<i>r.n.</i>	recessus neuroporicus
<i>ep.</i>	epiphysis	<i>r.m.ext.</i>	recessus neuroporicus externus
<i>f.bulb.</i>	formatio bulbaris	<i>r.p.</i>	recessus praeopticus
<i>f.i.</i>	foramen interventricu- lare	<i>som. area.</i>	primordial somatic cor- tex
<i>g.hab.</i>	ganglion habenulae	<i>tr.taen.</i>	tractus taeninae
<i>hem.</i>	lateral lobe of forebrain	<i>tr.th-cort.</i>	tractus thalamo-corti- calis (somatic)
<i>inf.</i>	recessus infundibuli	<i>v.tr.</i>	velum transversum
<i>l.s.</i>	lamina supraneuroporica		
<i>l.t.</i>	lamina terminalis		
<i>m.</i>	point of attachment of the membranous tela to the massive lamina supraneuroporica		

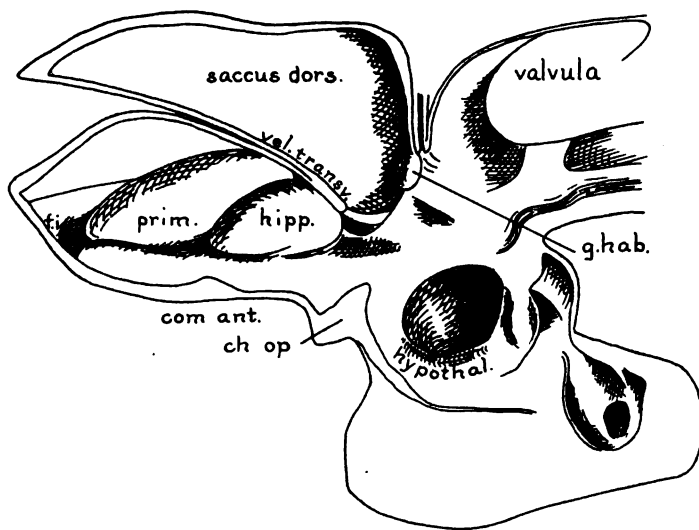


FIG. 2. Sketch of the right half of the fore part of the brain of the sturgeon as seen from the medial surface. The primordium hippocampi is bounded below by the sulcus Monroi. The velum transversum is attached to the brain wall behind the so-called praethalamus.

up the neural tube tapers to a point in the preoptic recess, and the lamina terminalis is the anterior part of the seam of closure along the mid-dorsal line (fig. 1). The conception of a "frontal Hirn-naht" of His is fundamentally wrong, but its convenience in descriptive anatomy has led to its continued use. The writer has at times inadvertently used or implied it, although he has held for some years the view here expressed. This view has been expressed by other workers also. The two views are incompatible and that of His is inconsistent with the facts of embryology and phylogenesis of the forebrain.

It is necessary to define more exactly how the velum transversum indicates the boundary between the diencephalon and telencephalon. The velum is a transverse in-folding of the tela chorioidea and owing to the arched form of the membranous roof in most vertebrates the lateral borders of the velum may be compared to the pillars of an arch. Where these pillars of the velum meet the lateral nervous walls of the brain in the embryo, these walls are indented by a vertical groove. This groove marks the boundary between the diencephalon and telencephalon. In the adult this boundary is marked by the attachment of the pillars of the velum to the massive lateral walls. In the brains of many fishes the arch of the velum is inclined forward so that the boundary line in question is not marked by the position of the velum in median sagittal section but by the place of attachment of the velum to the lateral wall. Fig. 2 shows what is meant in the case of the brain of the sturgeon, and the relations of the velum will be amply illustrated in the later papers of this series.

The features of the telencephalon in which we are chiefly interested are (1) the degree of evagination of the lateral lobes and the functional areas contained in these in different classes of vertebrates; (2) the area from which the hippocampal formation of higher brains is derived; (3) the area from which the general cortex is derived; and (4) the morphological position and value of the pallial commissures. These features will be reviewed very briefly by the aid of simple schematic figures.

The treatment of the cortex assumes the principle stated in a previous paper (1909, pp. 518-525) that the cortex develops from

centers in primitive vertebrates which serve for the correlation of incoming impulses of different kinds. The cerebral cortex everywhere is nothing else than a complex of correlation centers.

#### CYCLOSTOMES

In cyclostomes the evagination carries out the formatio bulbaris and the secondary olfactory centers in larger part. Indirectly, owing to crowding from in front, the wall of the telencephalon medium is folded so as to enter into the caudal wall of the lateral lobe. This is a part of the region heretofore named "striatum." It is not a proper part of the evagination and the folding spoken of accounts for the bifurcation of the lateral ventricle (fig. 4). The telencephalon medium includes secondary olfactory centers below and in front of the foramen interventriculare, so-called striatum below and behind the foramen, and primordium hippocampi (heretofore called "epistriatum") above the foramen. (See figs. 3 and

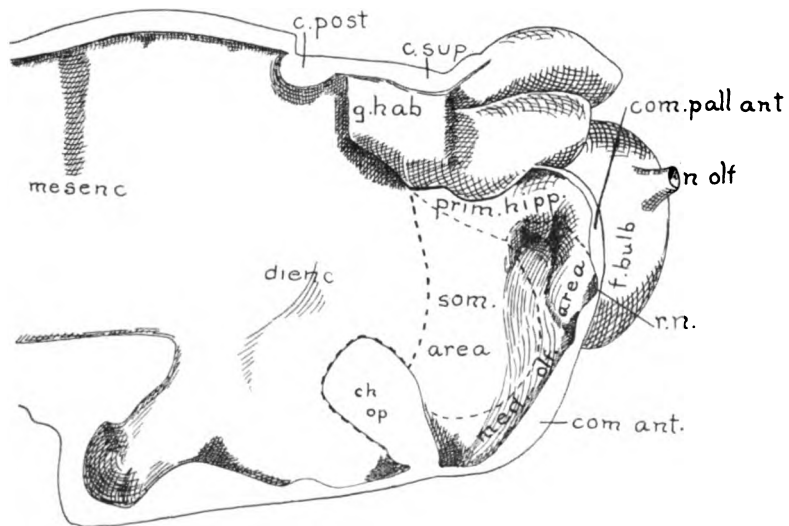


FIG. 3. *Petyromyzon dorsatus*, late ammocetes. Sketch of left half of fore part of brain to show functional areas in the telencephalon. The heavy broken line marks approximately the boundary between the telencephalon and diencephalon.

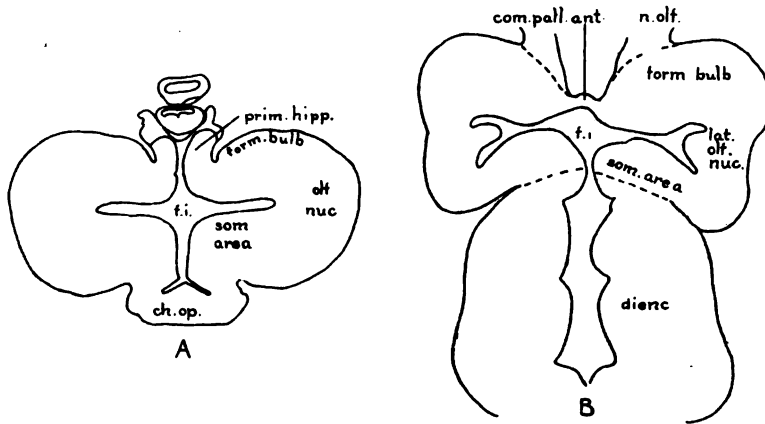


FIG. 4. *Petromyzon dorsatus*, late ammocetes. *A*, transverse section of telencephalon; *B*, horizontal section, both through the foramen interventriculare. The broken line beneath *som. area* in *B* marks approximately the boundary between diencephalon and telencephalon. The region labeled somatic area has heretofore been called striatum.

4.) That this primordium hippocampi belongs to the telencephalon and not to the diencephalon as held by Edinger, Sterzi, Tretjakoff and others is shown by the attachment of the velum transversum to the brain wall between this body and the ganglion habenulæ. The primordium hippocampi presents certain definite histological characteristics which are constant in fishes and amphibians. It receives olfactory fibers of the second order by way of a decussation in the lamina supraneuroporica and receives ascending fibers from the hypothalamus, the tractus pallii. The hypothalamus is a tertiary visceral and gustatory center in other fishes and amphibians and may be supposed to have similar functions in cyclostomes. The entrance of the tracts mentioned into the primordium hippocampi constitutes it a correlating center for olfactory and visceral impulses. It would thus furnish the starting point for the differentiation of the hippocampus and may possibly contribute to the formation of other structures.

The area called striatum is probably the beginning of the general or somatic cortex, but requires further investigation.

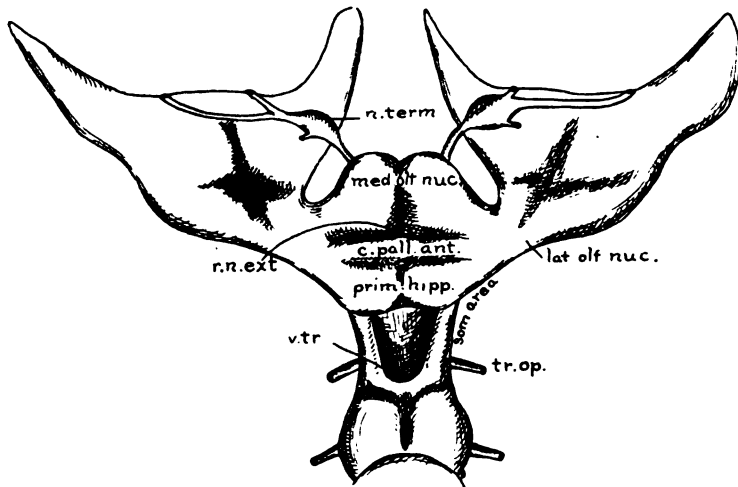


FIG. 5. *Scyllium stellare*, anterior part of the brain seen from above. The medial olfactory nuclei are very large, and the primordium hippocampi and anterior pallial commissure are carried far back. The line *r.n.ext.* marks the dorsal opening to the sagittal fissure. The line *v.tr.* marks the point of attachment of the velum transversum.

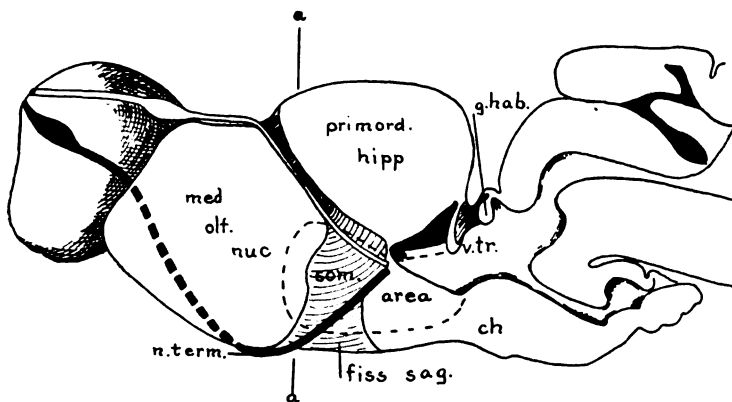


FIG. 6. *Scyllium stellare*, sketch of right half of fore part of brain. The telencephalic areas are lettered. The course of the nervus terminalis in *Scyllium* is shown in black. In light outlines the dorsal course of the nerve as seen in *Hexanchus*, *Squalus* and others. In *Scyllium*, instead of a slender external neuroporic canal there is a narrow fissure open both dorsally and ventrally. The somatic area is on the lateral surface. Its outline is projected upon the sagittal plane.

The anterior commissure lies in the lamina terminalis and connects the striatal areas. The commissure differs greatly in size in different species of petromyzontes and its constitution is not well understood.

The hippocampal primordia occupying the dorsal part of the walls of the unpaired ventricle converge forward and meet in a slight thickening of the lamina supraneuroporica (figs. 3 and 4). In this thickening are two kinds of fibers: (1) fibers crossing from the formatio bulbaris of one side to the primordium hippocampi of the other side, and (2) fibers which connect directly the evaginated portion of the so-called striatum. The important thing to notice here is that a decussation and a true commissure are found here *in the pallial position*, that is, above the neuroporic recess.

#### SELACHIANS

In selachians the evagination of lateral lobes has gone farther than in cyclostomes. Especially, the lateral ventricles are longer, the olfactory bulbs are carried out away from the secondary centers. The primordium hippocampi extends out some distance as the roof of the lateral ventricle, invades the lamina supraneuroporica as a great gray mass in which the pallial commissures lie and extends along the upper border of the wall of the unpaired ventricle (figs. 5, 6 and 7). The wall of the telencephalon medium is largely made up of a somatic correlation area, the beginning of the general cortex. That this area is wrongly assigned to the diencephalon by Edinger and others is indicated by the attachment of the velum just in front of the ganglion habenulæ (figs. 5 and 6).

The primordium hippocampi occupies the massive roof and is separated from the medial olfactory nuclei by the external neuroporic recess and a cell-free zone, the zona limitans (fig. 7). It receives from in front fibers of the olfactory tract (secondary) and direct and crossed fibers from both medial and lateral olfactory nuclei (tertiary fibers). The crossed fibers decussate in the lamina supraneuroporica where it is greatly thickened by the primordium hippocampi itself. Also, a large tractus pallii comes up from the hypothalamus to the primordium hippocampi as in cyclostomes. A part of this tract is uncrossed, a part crosses in the

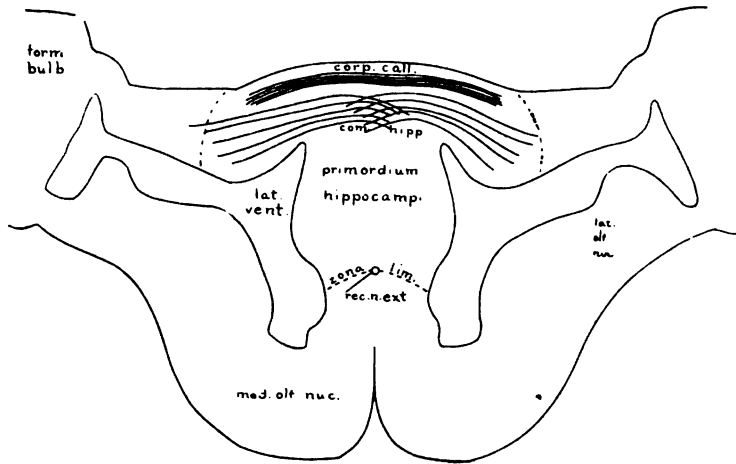


FIG. 7. *Scyllium stellare*, schematic transverse section of the telencephalon through approximately the line *a-a* of Fig. 6. The lateral ventricle is reconstructed from a number of sections before and behind this line.

postoptic decussations. The primordia of the two sides are connected by true commissural fibers in the anterior pallial commissure (fig. 8). Finally, that portion of the primordium which extends along the telencephalon medium is traversed by great numbers of fine fibers which cross in the superior commissure and constitute a true posterior pallial commissure of the hippocampal primordia (fig. 8).

From the primordium hippocampi fibers collect forward and descend through the medio-rostral and ventral wall between the lateral ventricles and go to the hypothalamus. They form definite bundles which are undoubtedly homologous with the fornix (fig. 8).

The existence of a somatic correlation center in the telencephalon is indicated by the presence of large numbers of fibers in the basal bundle which connect this area with the centers of the lemniscus and of the optic tracts in the thalamus. A descending path extending from this somatic area to the ventral part of the thalamus and to the motor centers forms part of the basal bundle described many years ago by Edinger. From the somatic area

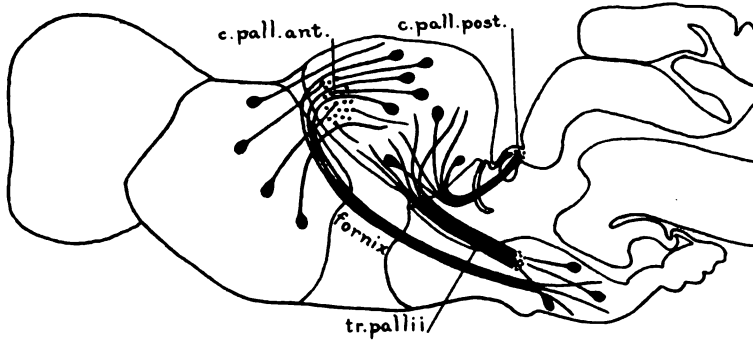


FIG. 8. Scheme of fiber tracts connected with the primordium hippocampi in selachians, based on *Scyllium*.

arises a large part of the fibers going to the nucleus habenulæ. These fibers may be given the name *tractus tæniæ* and are to be sharply distinguished from the fibers arising from olfactory centers, which should be called *tractus olfacto-habenularis*. Special tracts serving for correlation between somatic and olfactory centers will be described in a later paper. The somatic areas of the two sides are connected by a true commissure which crosses in the lamina supra-neuroporica above (ectal to) the commissura hippocampi (figs. 7 and 9). This commissure has the essential morphological and functional relations of a *corpus callosum*.

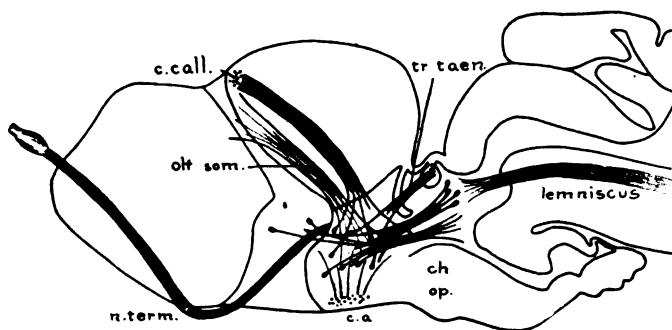


FIG. 9. Scheme of fiber tracts of the primordial somatic cortex in selachians, based on *Scyllium*. The ascending and descending fibers between thalamus and somatic cortical area occupy the middle of the figure and are not lettered.



The nervus terminalis enters the forebrain in selachians at a point immediately below and lateral to the neuroporic recess, and its fibers are directed toward the somatic area. This is the true or internal origin of the nerve as far as now understood. In some forms the nerve reaches this point by entering the dorsal surface and running down in the walls of the external neuroporic recess. In other forms it enters below and runs up to the same point. This difference is due to the secondary fusion of the medial olfactory regions which, apparently, has in some cases proceeded from below upward and pushed the nerve to the upper surface, in other cases proceeded from above downward and pushed the nerve to the lower surface (fig. 6).

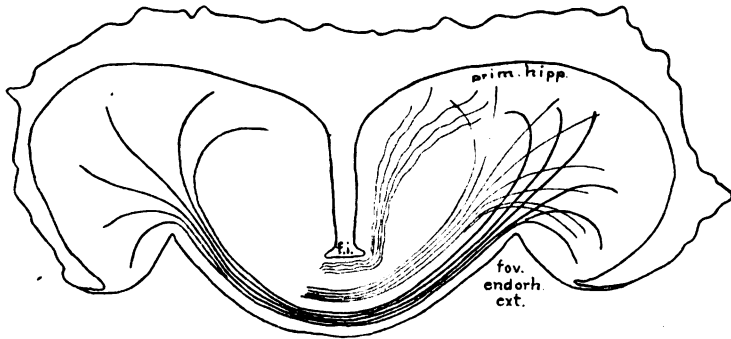


FIG. 10. *Amia* calva, schematic transverse section through the anterior commissure. The crossed olfacto-cortical tracts are shown above, the true commissural fibers of the primordium hippocampi below. The letters *f.i.* mark the sulcus (Monroi) which indicates the line of evagination and the position of the foramen in typical evaginated brains.

#### GANOIDS AND TELEOSTS

In these forms the evagination of lateral lobes has proceeded only so far as to form the olfactory bulbs which enclose lateral ventricles. All the rest of the forebrain constitutes a telencephalon medium whose walls are more or less everted as has been well described by Mrs. Gage and by several later writers (fig. 10). In the ventricular surface of each wall can usually be seen a sulcus Monroi which leads forward to the lateral ventricle (figs. 2, 10 and 11).

This sulcus marks the line of evagination and the position of the foramen interventriculare in those forms whose forebrains are evaginated.

Above this sulcus the ventricular surface of the lateral wall is covered to a variable depth by the peculiar tissue which constitutes the primordium hippocampi. Laterally this body is separated from the somatic correlation area by a sulcus somewhat below the line of the tænia (fig. 10).

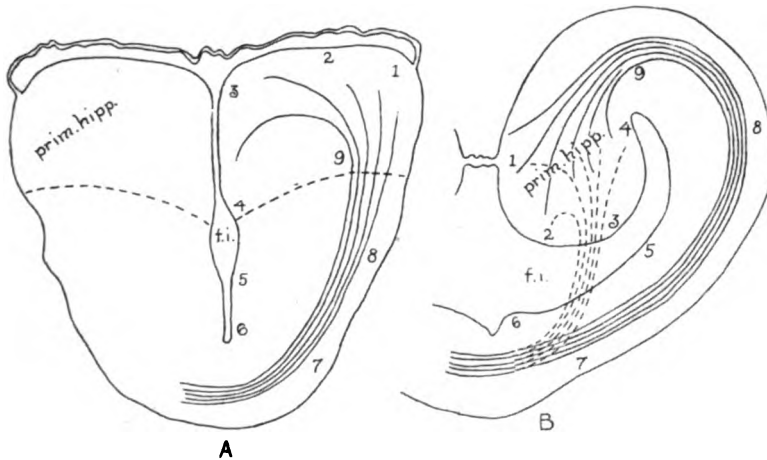


FIG. 11. Sketches for comparison of everted and evaginated types of forebrain. A, transverse section of forebrain of young *Amia* (25 mm.); B, diagram to show the translocation of parts that would take place in the evagination of such a brain. The course of the commissure is indicated by continuous lines. In B, the broken lines show the actual course of the "commissura hippocampi" behind the lateral ventricle in amphibians.

From in front the primordium hippocampi receives olfactory fibers of the second and third orders, many of which decussate in the anterior commissure complex. From behind, the large tractus pallii comes up from the hypothalamus, partly decussating in the anterior commissure (fig. 12). The hippocampal primordium although very different in form, has essentially the same relations as in cyclostomes and selachians.

The somatic area receives fibers from the lemniscus center in the thalamus, and probably from the tectum mesencephali (fig.

12). Fibers ascending from the lateral geniculate bodies have not yet been seen. In these forms also there is a clear distinction between the tractus tæniæ and the tractus olfacto-habenularis.

Ganoids and teleosts possess no true anterior pallial commissure. The fibers connecting both the hippocampal and the somatic cortical primordia pass by way of the anterior commissure complex. A detailed discussion of this fact and its connection must be

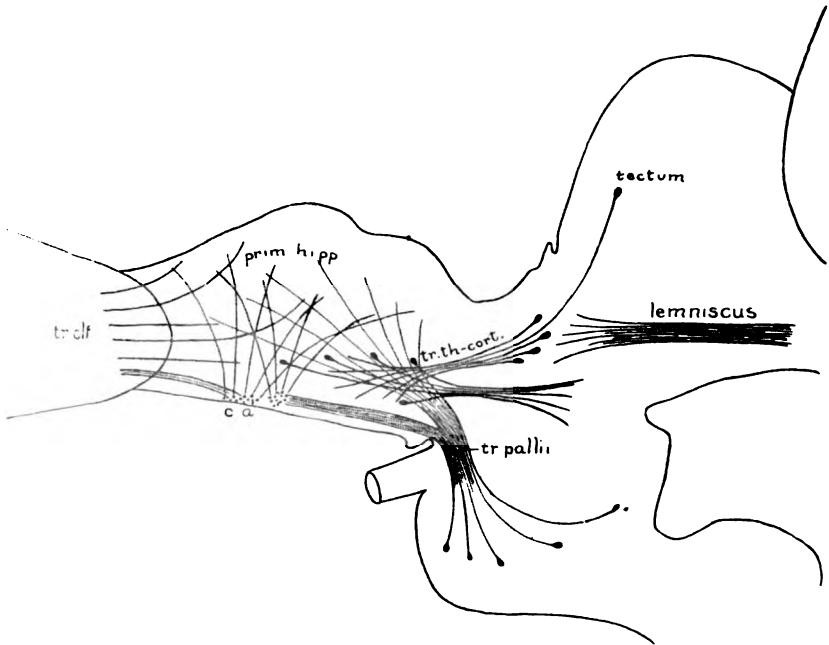


FIG. 12. Scheme of fiber tracts connected with the primordial cortex of the sturgeon.

postponed, but it should be held in mind that the commissures of the cortical areas cross beneath the unpaired ventricle, then pass lateral, external and caudal to the foramina and lateral ventricles and so into the everted areas which correspond more or less completely to the roof of brains of the evaginated type (figs. 10 and 11).

## AMPHIBIANS

The evagination of the lateral lobes has gone much farther and is almost complete. The elongation of the forebrain has taken place not at the olfactory peduncle as in many selachians and teleosts, nor at the telencephalon medium as in some selachians and especially in *Chimæra*, but in the region between the foramen interventriculare and the olfactory peduncle. The primordium hippocampi has been nearly all evaginated and forms the upper part of the medial wall of the vesicular lateral lobe, together with an undetermined part of the dorsal wall. In urodeles a small part of it remains in the dorsal part of the wall of the un-

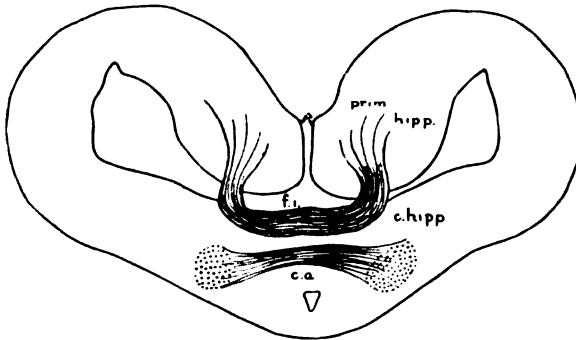


FIG. 13. *Necturus maculatus*, transverse section through the foramen interventriculare. The section is seen from the caudal surface and the commissure related to the hippocampus is represented as passing up lateral to the foramen interventriculare and caudal to the lateral ventricle to reach the hippocampus.

paired ventricle as in cyclostomes. A zona limitans separates the hippocampal area from the medial olfactory area, which occupies the lower part of the medial wall ("septum") and extends into the floor of the unpaired ventricle where it forms the "precommissural body" of Elliot Smith (fig. 14).

The evagination has involved a greater or less part of the somatic area and has been prolonged backward to form a posterior pole. The relations of the hippocampal and somatic areas in this pole are still in doubt.

A partial study of the fiber tracts in several amphibians has shown in connection with the primordium hippocampi fibers from the olfactory centers, an ascending tractus pallii from the hypothalamus and a fornix descending to the hypothalamus as in selachians. The lateral forebrain bundle contains many ascending somatic sensory fibers which end in the lateral parts of the hemisphere. According to Herrick there are present in the frog fibers from the lemniscus centers in the thalamus, optic radiations and auditory radiations. The amphibian brain seems to the writer to present a high degree of complexity and specialization of

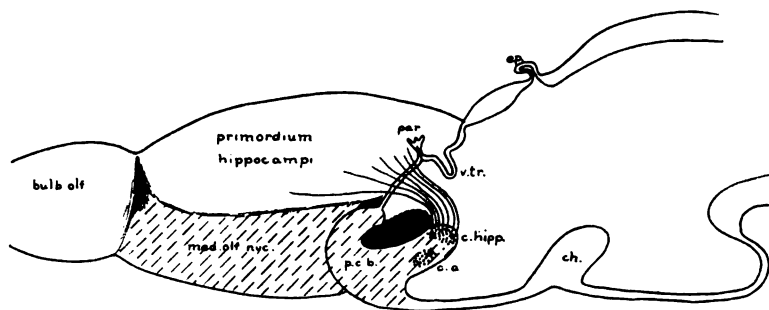


FIG. 14. *Necturus*, sketch of right half of forebrain seen from the medial surface. The medial olfactory nucleus, precommissural body and bed of the commissures are shaded by short oblique lines. A part of the precommissural body extends up over the foramen interventriculare. The commissure of the primordium hippocampi runs up behind the foramen interventriculare (compare fig. 15).

structure based upon an apparently simple and primitive arrangement of the neurone-bodies in a central gray. More detailed studies of the fiber connections are necessary to enable us to determine the extent and boundaries of the several functional areas, especially the hippocampal and somatic cortical areas and the pyriform lobe.

The forebrain commissures of amphibians closely resemble those of ganoids and teleosts. In the lamina terminalis are found an anterior commissure chiefly related to the lateral basal bundle and the somatic correlation areas, and a so-called hippocampal commissure (fig. 13). The latter crosses beneath the unpaired ven-

tricle, rises lateral and caudal to the interventricular foramen and bends forward over the proximal part of the lateral ventricle to enter the medio-dorsal or hippocampal area. The disposition of this commissure stands in sharp contrast with that of selachians and of reptiles and mammals and agrees in all essentials with that of ganoids and teleosts. When Osborn ('88) interpreted this commissure as the corpus callosum he recognized the great difficulty presented by its position. The writer has pointed out ('02, '06) that this commissure can not be compared morphologically with the psalterium of mammals. The neglect of this by all recent students of the amphibian brain, and especially the failure to observe the difference in position of the commissures in amphibians and reptiles must lead to confused ideas of forebrain morphology. Compare figs. 14 and 15.

From the posterior pole of the hemisphere a large posterior pallial commissure accompanies the tractus tæniæ to cross in the superior commissure as in selachians.

#### REPTILES AND MAMMALS

From the work of Elliot Smith, His, Ziehen, Zuckerkandl and others the general relations in the reptilian and mammalian brain are well understood. The hippocampal area occupies the medio-dorsal region of the hemisphere in reptiles and in the series of mammals is modified, owing to the growth of the general cortex and corpus callosum, until in man the only well developed hippocampal formation lies between the splenium of the corpus callosum and the tip of the temporal lobe (uncus). From the somatic area of fishes and amphibians the general cortex has developed between the hippocampus dorso-medially and the pyriform lobe laterally. A part of the somatic area surrounding the lateral basal bundle has differentiated into the corpus striatum through which the enlarged lateral basal bundle runs as the internal capsule.

The disposition of the commissures is of great importance for an understanding of the comparative morphology of the telencephalon. The anterior commissure serves to connect the olfactory

areas and probably contains somatic elements in addition. In mammals two pallial commissures are present, the commissura hippocampi and the corpus callosum; in reptiles the corpus callosum is not yet certainly known. A posterior pallial commissure is present in some reptiles but is unknown in mammals.

In reptiles the hippocampal commissure crosses rostral to the unpaired ventricle. Its arms rise up in front of the interventricular foramina in the medial walls of the lateral ventricles, to enter the hippocampal area directly (fig. 15). In lower mammals the hippocampal commissure has the same position as in reptiles and in higher mammals is carried up over the third ventricle by the upward and backward growth of the hemispheres.

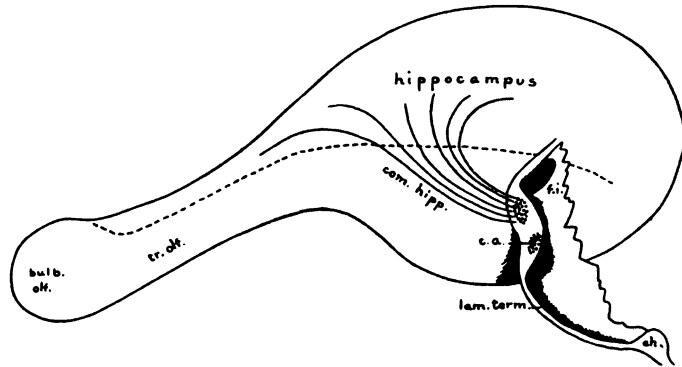


FIG. 15. Sketch of the right half of the forebrain of a reptile to show the relations of the hippocampus and of its commissure. The commissure goes up in the medial wall in front of the foramen interventriculare.

An interesting question arises concerning the position of this commissure with reference to the neuroporic recess. This question does not involve the main facts regarding the history of the hippocampus and its commissure in mammals as set forth by Elliot Smith and others, nor does it affect the question at issue between Smith and His as to the primary or secondary character of the fusion which constitutes the "commissure bed." The question is whether the commissure lies in the lamina terminalis (*below* the neuroporic recess) or in the lamina supraneuroporica (*above* the

neuroporic recess). In reptiles the hippocampal commissure lies more or less close to the anterior commissure so that in late embryos and adults the relations of the neuroporic recess are obscured. In lizards it is probably represented by the recessus inferior of Elliot Smith (fig. 16). The early development shows that the neuroporic recess is situated close in front of the anterior commissure, that above the recess is a thickened part of the lamina supraneuroporica related to the hemispheres; the remainder of this lamina is a membranous tela in which appears the paraphysis just in front of the velum transversum (fig. 17). As the hemispheres

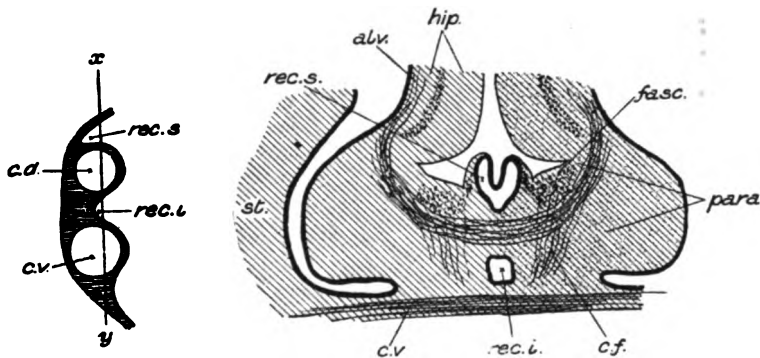


FIG. 16. A portion of a transverse section through the brain of a monitor (*Hydrosaurus*). From G. Elliot Smith. In the figure to the left the line *x-y* shows the plane of the section. *alv.*, alveus; *c.f.*, columna fornicis; *fasc.*, fasciculus marginalis; *hip.*, hippocampus; *para.*, paraterminal body; *rec. i.*, recessus inferior; *rec. s.*, recessus superior; *c. d.*, hippocampal commissure; *c. v.*, anterior commissure.

develop the recessus neuroporicus is reduced or obliterated and the hippocampal commissure comes to lie close to the anterior commissure.

In mammals the neuroporic recess is a prominent pit in front of the anterior commissure in early stages, but the growth of the hemispheres and commissure-bed reduces it to a shallow pit in the same position (figs. 18 and 19). The thickened lamina which constitutes or contains the bed of the hippocampal commissure is the lamina supraneuroporica and extends laterally into the hemispheres as in reptiles. When the hippocampal commissure ap-



pears it is widely enough separated from the anterior commissure so that there can be no doubt that it lies above the neuroporic recess in the lamina supraneuroporica (figs. 18 and 19).

In reptiles and mammals the commissura hippocampi and corpus callosum are said to be embedded in the precommissural body (Elliot Smith and others). This precommissural body is formed by the invasion of the lamina terminalis by the medial olfactory nuclei or by the secondary fusion of these nuclei. Such a fusion is most extensive in selachians where the nuclei are very volumi-

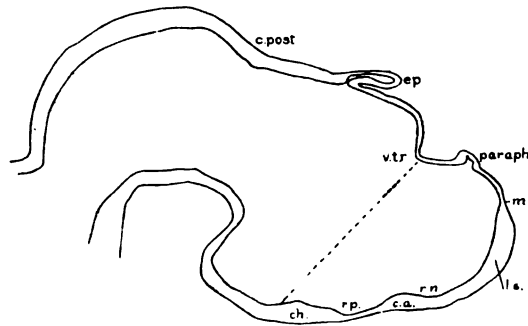


FIG. 17. Median sagittal section of the brain of a turtle embryo at the time of formation of the paraphysis. The broken line marks the di-telencephalic boundary. At *m* is the caudal margin of the nervous part of the lamina supraneuroporica. The tela behind this is not only thin but differs histologically from the lamina supraneuroporica. The neuroporic recess has been traced in earlier stages and undoubtedly lies between the parts identified in this figure as the anterior commissure and the lamina supraneuroporica.

nous. In some mammals also this fused body is very large. So far as my observations go the corpus callosum in mammals is situated along the line of the zona limitans, while the hippocampal commissure is involved or enwrapped to a variable extent in the precommissural body. I can harmonize this with the condition in selachians and with the facts of development in mammals above cited only on the supposition that the precommissural body has secondarily pushed up into the region of the lamina supraneuroporica, probably following along the system of olfacto-hippocampal fibers.

The bearing of these facts upon the phylogeny of the cortex and its commissures is seen when the brain of the mammalian embryo is compared with the selachian brain (fig. 20). The pallial areas in selachians, reptiles and mammals meet in the lamina supraneuroporica which is thickened by invading gray matter and serves for the passage of the hippocampal commissure and corpus callosum. The cyclostomes present the same condition, but with simpler commissures. The ganoids, teleosts and amphibians present a very

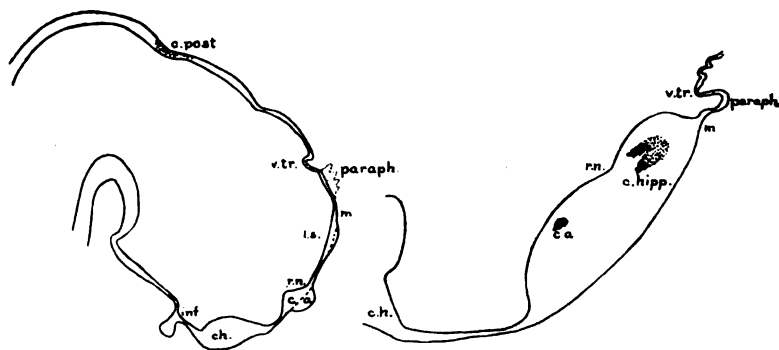


FIG. 18. Median sagittal sections of the forebrain in cat embryos to show the relations of the commissures to the recessus neuroporicus. To the left a 12 mm. embryo (Minot collection No. 400, slide F., sec. 3/6); to the right, a 31 mm. embryo (Minot collection No. 527, slide Bk, sec. 1/3). This section of the 12 mm. stage is slightly oblique. The paraphysis is drawn in dotted outline as it appears in adjacent sections. The slight thickening at *l. s.*, shows that the lamina supraneuroporica is passing over into the medial wall of the hemisphere. The point *m* corresponds to the point so lettered in the turtle embryo. This is about the point at which the recesses neuroporicus has been placed by previous authors. The nearness of this to the paraphysis is sufficient evidence that the lamina terminalis does not extend up to this point. The figures are from free-hand sketches but the relations are essentially correct.

different condition, in which the fibers analogous to the hippocampal commissure and corpus callosum, so far as such fibers exist, run with the anterior commissure in the lamina terminalis. It seems probable that the pallial commissures maintain a true pallial position in cyclostomes, selachians, reptiles, birds and mammals (and possibly dipnoans), while in ganoids, teleosts and

amphibians they have a peculiar disposition the origin of which requires to be explained.

The features in the author's account of the evolution of the cerebral cortex which are distinctive are the following:

1. The telencephalon possesses an unpaired ventricle whose walls constitute a very important part of the forebrain (telencephalon medium).
2. The telencephalon of primitive vertebrates possesses visceral and somatic correlating centers which are the primordia of the hippocampal formation and general cortex respectively.

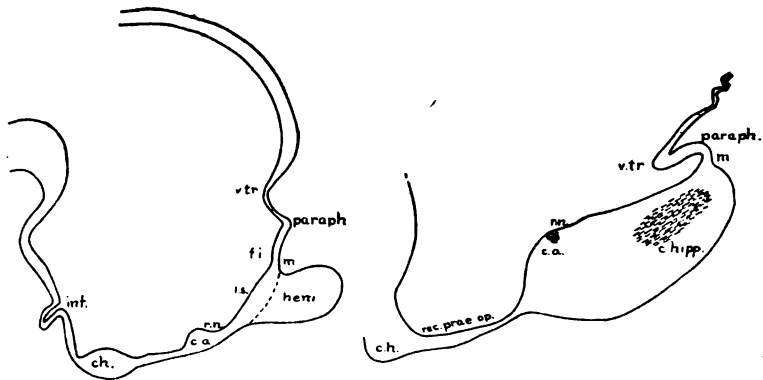


FIG. 19. Sagittal sections of the forebrain in rabbit embryos to show the relations of the commissures. To the left an embryo of 14 days, 10.5 mm. (Minot collection No. 156); to the right an embryo of 20 days, 29 mm. (Minot collection no. 171.) Drawn as fig. 18.

These correlating centers are equally old (unless the somatic be the older) and the terms archipallium and neopallium are inappropriate. In their stead should be used some such terms as visceral pallium and somatic pallium.

3. These cortical primordia are at first not involved in the evagination of lateral lobes, but lie in the wall of the unpaired ventricle. They are gradually evaginated into the lateral lobes in selachians, dipnoans, amphibians and reptiles.

4. The primordium of the visceral cortex is defined by (1) the entrance of olfactory fibers of the second and third orders, (2) the

entrance of an ascending tract from the hypothalamus of general visceral or gustatory function or both, (3) the possession of a true commissure in the lamina supraneuroporica and another in the superior commissure (anterior and posterior pallial commissures), (4) the presence of true fornix, and by (5) peculiar histological structure. With regard to the commissures, the cyclostomes require further investigation, the ganoids, teleosts and amphibians present a peculiar modification of the anterior pallial commissure and the mammals apparently lack a posterior pallial commissure.

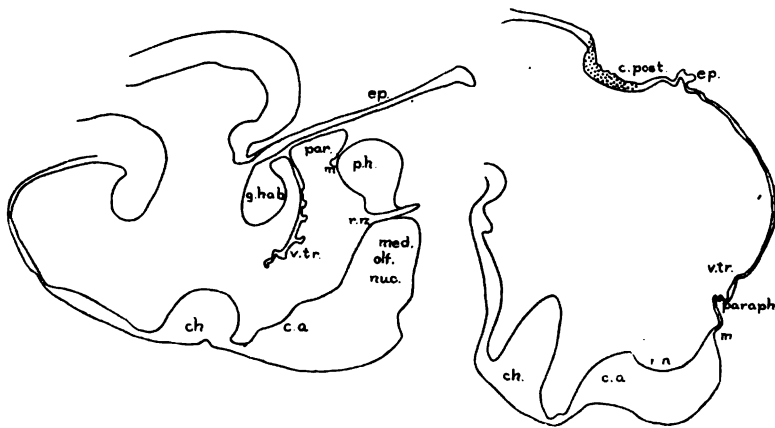


FIG. 20. Sketches for comparison of the human embryonic forebrain with that of the selachian. On the left a sagittal section of the forebrain of *Squalus acanthias* of about 70 mm., from a preparation in the Minot collection. On the right a median sagittal section of the forebrain of a human embryo (Minot collection no. 181, slide A1. sec. 1/3). Drawn as fig. 18.

5. The primordium of the somatic cortex is defined by (1) the entrance of ascending fibers from the thalamus (and tectum mesencephali) chiefly carrying cutaneous, visual and muscle-sense impulses (exteroceptive and proprioceptive centers), (2) giving origin to descending fibers to the thalamus and probably medulla oblongata and spinal cord (Van Gehuchten and others), and (3) the possession of a true pallial commissure (corpus callosum) in the lamina supraneuroporica except ganoids, teleosts, and amphibians. The writer does not accept Edinger's hypothesis that the oral sense

centers in the telencephalon play the chief rôle in the origin of the cerebral cortex. The area recognized by the writer as the primordium of the somatic cortex has been assigned by all recent authors to the diencephalon and is wholly distinct from any center which Edinger has suggested for his oral sense. A cutaneous nerve of the first head segment (*nervus terminalis*) probably entered the primordium of the general cortex, but this is not an *essential* part of the account here given.

I wish to express my best thanks to Dr. C. S. Minot for the opportunity to study his valuable collection of mammalian embryos.

# ON THE GENESIS OF AIR CELLS IN THE CONCHÆ NASALES<sup>1</sup>

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WITH SEVEN FIGURES.

Since the time of Santorini, who apparently was the first anatomist to call attention to the cavities frequently found in the middle nasal conchæ, many conflicting and erroneous hypotheses have been advanced as to the nature and origin of these spaces. Many of the theories are from the pens of clinicians who removed at operation portions of the walls of such cavities that were changed by pathological processes, and then attempted to explain the origin of these spaces from the altered tissue removed.

It is the purpose of this paper to briefly analyze some of the theories extant and, if possible, arrive at the true origin and nature of these cavities—basing the conclusions on a study of the lateral wall of the nasal cavity in the fetus, child, and adult.

These cavities have been variously termed cysts, abscesses, osseous cysts, exostoses, neoplasms, ectasias of the ethmoid bone, air cells, aberrated ethmoid cells, and when large and occurring in the ventral portion of the conchæ mediæ, as conchæ bullosæ.

I have found these cavities, which I consider as normal ethmoid cells, in the concha media, processus uncinatus, and agger nasi. Schaeffer reports an isolated case in the concha inferior, and Bayer found one in the concha superior. The usual seat, however, for these cells is in the anterior half of the concha media, and it is to the cells in this position that the above terms have been applied by various writers. It is not common to find cells in the adult superior conchæ. The reason for this is evident

<sup>1</sup>Presented at the 25th session of the Amer. Assoc. Anat., Boston, December, 1909.

when we remember that the adult superior ethmoidal conchæ are as a rule merely thin lamellæ of bone covering medially the posterior group of ethmoid cells. The most prominent conchæ in this position are due to large posterior ethmoid cells which push the median walls of the cells toward the cavum nasi; thus forming more pronounced swellings, therefore seemingly larger superior ethmoidal conchæ. It must, however, be remembered that the ethmoidal conchæ are primarily solid appendages of the lateral ethmoidal masses. The developing posterior group of ethmoid cells do not only extend into the lateral ethmoidal masses, but also into the superior ethmoidal conchæ. This extension progresses until the conchæ are represented finally by mere thin lamellæ of bone, covered and lined with mucous membrane. In this sense, portions of posterior ethmoid cells are always conchal cells, just as are those which occupy the middle conchæ.

Conchal cells are practically unknown in the concha inferior, because the latter structure is too far removed from the seat of the modifications in the ethmoidal region consequent upon the formation of the ethmoidal fissures, the structures overhung by the concha media, and the Anlagen of the paranasal chambers.

Santorini (1739) in his "*Observationes Anatomicæ*" (p. 89), calls attention to cavities in the ventral end of the concha media and claims priority in describing them. He found the cavities both in cadavers and skeletons, but was unable to find ostia for these spaces. He suggests that his failure in finding openings for the cavities might be due either to the softness of the mucous membrane or smallness of the apertures, and concludes that his successors may find ostia for these spaces. He says: "*Ejus tamen inveniendi alterius erit otii, et sedulitatis opus.*"

Depuytren ('30) speaks of them clinically under the title of "*Kyste à parois osseux pris pour un polyp fibro-celleux,*" and Chantreuil ('69) examined and described a specimen under the title of "*Exostose Celluleuse des Fosses Nasales.*"

Glasmacher ('84) writes on "*Knochenblasenbildung in der Nase,*" and describes a cell 22 mm. long and 18 mm. wide. He says:

Was nun die Knochenblasenbildung im Bereiche der Muscheln angeht, so bemerke ich vorab, dass ich diese ebenso wenig, wie im Siebbein für pathologisch ansehe; ihre Wichtigkeit liegt nur in ihrem abnormen Wachsthum.

Schaeffer ('85) speaks of these cells and reports four cases one of which he found in the ventral end of the concha inferior—the only cell of this concha I have been able to find reported in the literature. I will again refer to this cell in a subsequent paragraph, in an attempt to explain its genesis. He considers the cavities of pathological origin, and in defence of his belief he offers the following:

Zudem gaben die Patienten an, dass sie seit Jahren nach Erkältungen eine Zunahme der Stenose des Nasenganges beobachtet hätten. Dieses Wachsen setzt doch gewisse Reize voraus, die es veranlassen, wenn anders die ganze Knochenblase nicht angeboren ist, was in meinen Fällen nicht der Fall war. Solche Processe müssen wir aber immer unter die pathologischen einrechnen.

He apparently fails to recognize that a normal ethmoidal cell developing in the concha media will gradually lessen the lumen of the nasal fossa. A conchal cell will always begin small, and develop just as the ethmoid cells of the lateral mass but in many cases remains of such size as to cause no trouble, and only when excessively large or diseased is the attention of the clinician directed to it—unless discovered accidentally when exploring this region of the nose for some other cause. The stimulus or irritant of which Schaeffer speaks causing the growth of these cavities is certainly not different from the stimuli which cause the formation of the other paranasal sinuses.

Macdonald ('91) writes on these cells under the title of, "On Cyst and Abscess of the Middle Turbinate Bone." In a previous discussion he "attempted to show that the development of such structures might be analogous to similar tumors of long bones," and in the article under the above title he concludes the theory as "hasty and incorrect"—then advances his osteophytic theory. Since the latter theory has found its way into many



articles, also into some text-books, it may not be amiss to quote briefly from his original paper.

The process in all probability begins in an osteophytic periostitis, a condition common in this region, and resulting in a general increase in the size of the bone in every diameter. The free margin being incurved upon itself, from the pathological process just mentioned, will bring it in contact and ultimately in union with the body of the bone. Thus a cavity may become enclosed and sealed at all points by a similar process occurring at the extremities." He further says: "That the above is the correct explanation of the remarkable neoplasms is proved by a microscopical examination of any portion of the cyst wall; on each side of the thin lamina of bone is found a layer of mucous membrane covered with columnar epithelium."

The theory as advanced by Macdonald seems fanciful and to my mind is certainly not the true explanation of the origin of these cavities. In some cases there is a slight lateral and superior curling of the free border of the concha media, thus increasing the extent of the so-called sinus of the concha media, but this sinus is in no way the homologue of conchal cells. Testut and Jacob also refer to this curling of the concha media with reference to cell formation.

According to my specimens, cells invariably have ostia communicating either with the anterior or posterior ethmoid cells, with the inferior ethmoidal fissure (meatus superior) or the infundibulum ethmoidale. Some cells communicate directly with the meatus medius under cover of the concha media. Lothrop's investigation of these cells fully confirms this. He says: "Every cell without exception possesses an ostium." In the second place, Macdonald's theory could not account for the cells having their ostia opening into the meatus superior, nor for those communicating with the infundibulum ethmoidale—yet in my cases fully one-half of the cells open in these positions. The only factor in defense of the theory is that the cavities are lined with columnar epithelium. However, this may be explained in a far more satisfactory manner if we consider the development of these cells as analogous to that of the ethmoid cells of the lateral masses. In fact very many of these cells are merely parts of other ethmoid cells (figs. 4 and 5).

Knight ('92) reported a case under the title of, "Cyst of the Middle Turbinate." He mentions two possible theories, viz: (a) the "result of a rarefying osteitis," (b) that of Macdonald as given above. In his text-book ('03) he says:

In the majority of cases it doubtless results from a rarefying osteitis inducing absorption of the interior of the body of the bone.

This latter theory is not permissible when we recall that these cells or cavities are lined with mucous membrane similar to that of the ethmoid cells of the lateral masses.

Reardon ('98), writes on "Osseous Cysts of the Middle Turbinate." He thinks the cavities are either ectasias of the ethmoid which, as the ethmoid develops, become separated from it completely or incompletely; or are aberrated ethmoidal cells which developed in the conchæ (Heymann's theory).

It is easily understood why many clinicians continually refer to these cavities as cysts, osseous cysts, abscesses, etc., because they generally have their attention directed to them only when they are diseased, unless the air cell becomes very large without disease and leads to symptoms of obstruction and pressure. They then attempt to explain the genesis of these primarily normal cells from the pathological condition found.

It must be remembered that these conchal cells, like any of the ethmoid cells or other paranasal sinuses, may become the seat of an empyema or mucocoele, and enlarge, because the ostia of these cells are invariably placed at the highest points of the cavities and very disadvantageously placed as drainage openings—a fact easily understood if we consider their development. The existence of air cells in the conchæ, etc., is certainly not the result of an empyema or rarefying osteitis, but because these cells are normally found in these positions, they may become the seat of pathological conditions just as any other cell of the ethmoid labyrinth.

Zuckerkindl refers to the distension, by an air cell, of the ventral extremity of the concha media as, "concha bullosa."

Lothrop after studying a large number of these cavities in adult specimens concludes that they are ethmoid cells—a view

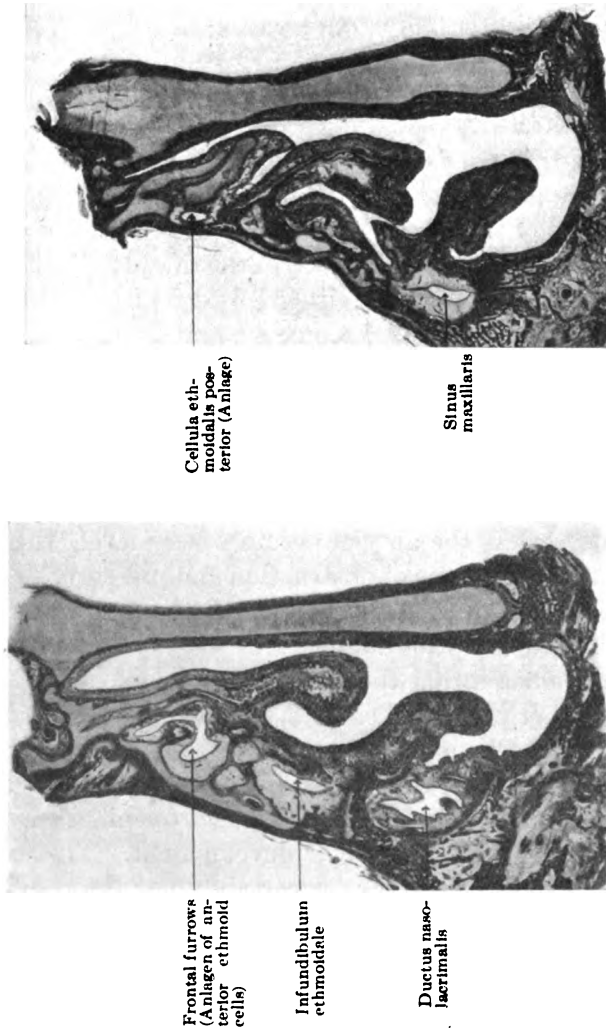


FIG. 1  
A B

FIG. 1. Frontal sections through the right half of the nose of a fetus aged about eight months, showing Anlagen of ethmoid cells. Section **A** is in the region of the anterior ethmoidal cells, and section **B** is in the region of the posterior group of ethmoidal cells.

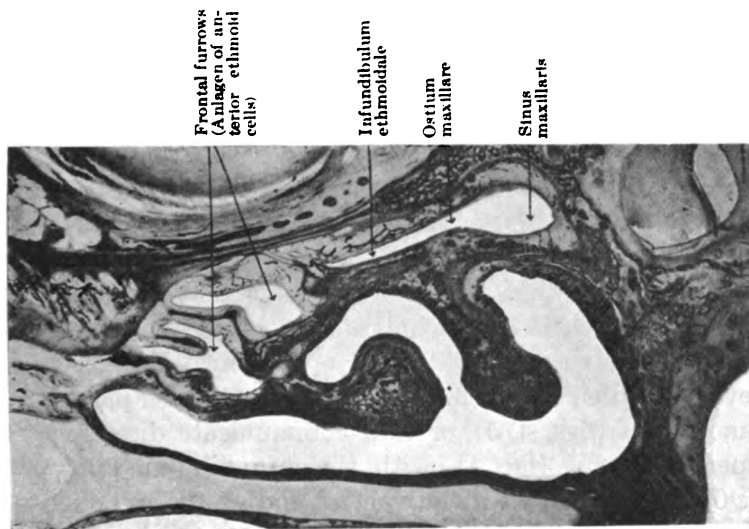


FIG. 2

FIG. 2. Frontal section of the left half of the nose of a fetus at term, in the region of the ostium maxillare. Note how some of the primitive ethmoidal cells tend to grow into the concha media.

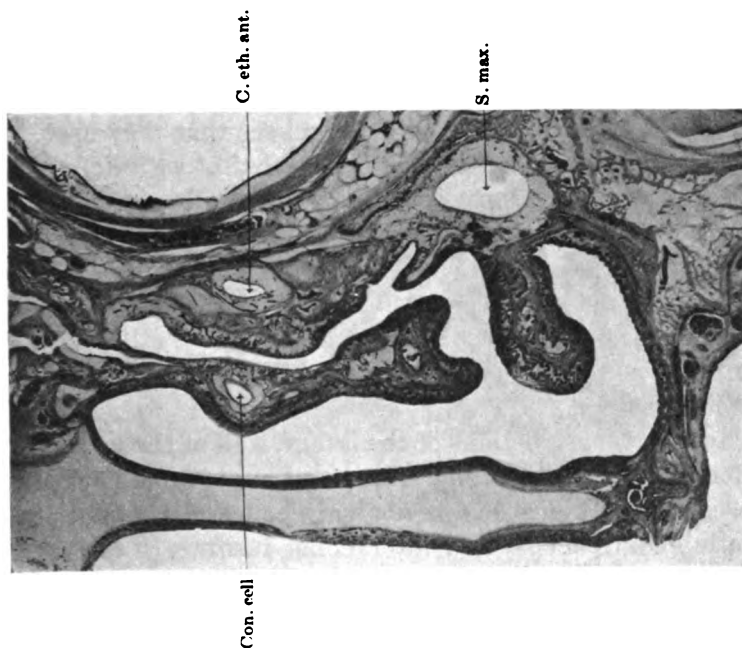


FIG. 3

FIG. 3. Frontal section from the same nose as that in Fig. 2. Note that the section is farther dorsal than that in the preceding figure. Note also the conchal cell in the concha media. The ostium of this cell communicated directly with the middle meatus, a few sections farther dorsally.

Con. cell, conchal cell; C. eth. ant., cellula ethmoidalis anterior; S. max., sinus maxillaris.

also held by Shambaugh, who speaks of them as "anatomical variations of ethmoid cells."

In order to better understand these cells and see that they are nothing other than ethmoid cells, it is essential that we briefly consider the origin of the ethmoid labyrinth. The location of these cells appears less abnormal when we recall that the ethmoidal conchæ and uncinæ processes are merely appendages of the lateral ethmoidal masses. There is no reason, therefore, why ethmoid cells should not at times, in the formation of the ethmoid labyrinth, grow into these appendages just as they grow into the lateral ethmoidal masses.

According to my reconstructions of the lateral wall of the nasal cavity of different aged fetuses, the primitive ethmo-turbinal fold, with its subsequent modifications is not only concerned in producing the ethmoidal conchæ and intervening furrows (meati), but also the structures operculated by the middle nasal concha: viz., the processus uncinatus, the bulla ethmoidalis, the hiatus semilunaris, and the infundibulum ethmoidale. These modifications are also intimately related with the Anlagen of the paranasal sinuses.

The posterior group of ethmoid cells are primarily constricted from, or are direct extensions of, the furrows separating the primitive ethmoidal conchæ, and the anterior group develop from the preformed accessory furrows of the middle meatus—hence are in relation with the grooving and structures found in this location.

Frontal sections of the fetal nose before birth (fig. 1) already indicate the Anlage of the ethmoid labyrinth, and at term it is comparatively well advanced (fig. 2). In this connection it is an interesting fact that the ostia of conchal cells invariably communicate directly or indirectly with the points at which the ethmoid cells developed their Anlagen. These cells are either parts of other ethmoid cells (figs. 4, 5), or they communicate directly with the superior meatus (fig. 4), with the infundibulum ethmoidale (fig. 6), or directly with the ventral end of the middle meatus (fig. 5). The ostia in the latter case are on the lateral wall of the concha media.

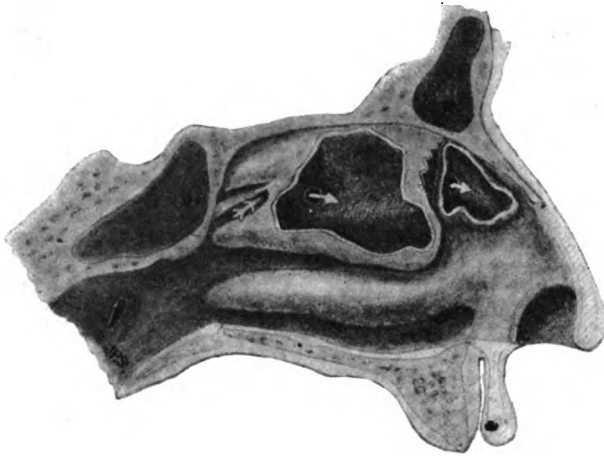


FIG. 4. Drawing from a specimen of the lateral nasal wall. The cell extending into the agger nasi and into the processus uncinatus communicates with the infundibulum ethmoidale. Note that the ethmoid labyrinth is largely replaced by a very large bullous cell extending into the concha media and communicating with the ventral extremity of the meatus superior.

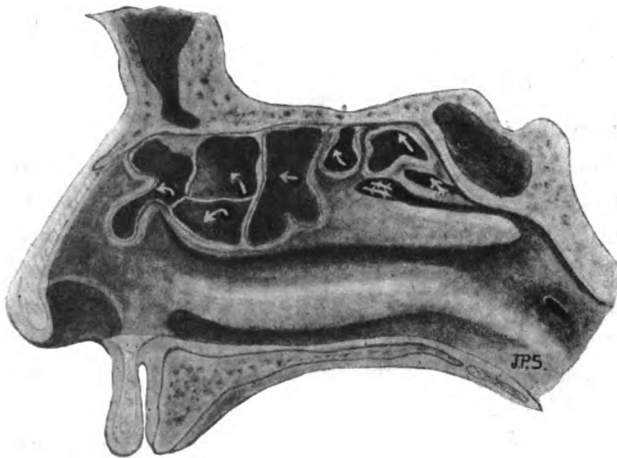


FIG. 5. Drawing from a specimen of the lateral nasal wall. The arrows indicate the ostia of the several cells. Note that one of the cells which communicates with the meatus superior extends into the concha media. Note also the cell in ventral extremity of the concha media with an independent ostium communicating directly with the meatus medius.

Although the Anlagen of the ethmoid cells are primarily constrictions from the nasal fossæ, yet the extension and development of these cells depend upon the simultaneous processes of growth (of the sacs) and resorption (of surrounding tissue). In this manner, as age advances, the cells extend farther and farther into the lateral masses of the ethmoid bone, and in the adult are completed by the articulation of the ethmoid bone with the frontal, lacrimal, sphenoid, maxillary, and palate bones. These developmental processes are doubtless in some cases carried further than in others, hence the extension of ethmoid cells not only farther into the lateral masses of the ethmoid bone, but also into its appendages, such as the ethmoidal conchæ and uncinate processes. Ethmoidal cells also at times extend into the agger nasi and encroach upon the sinus frontales and sphenoidales.

A reference to figs. 4 and 5 shows the extension of the inferior ethmoidal groove (meatus superior) not only into the lateral mass but also into the concha media, thus forming conchal cells which are merely parts of the lateral mass cells. At first thought it may seem difficult to account for the conchal cells having their ostia opening inferior to the attachment of the concha media, either into the middle meatus or the infundibulum ethmoidale (figs. 4, 5, 6). However, when we remember the great modifications of this portion of the middle meatus overhung by the concha media, consequent upon the formation of the structures found here, and that the anterior group of ethmoid cells have their origin in this position, it is not difficult to see how some of these cell-Anlagen may extend not only into the lateral mass of the ethmoid bone, but also into the uncinate process, the concha media, and the agger nasi (figs. 4, 5, 6). Fig. 3 shows a small cell already present in the concha media of a fetus at term. Of course most conchal cells must necessarily appear comparatively late in the formation of the ethmoid labyrinth, since the positions they occupy with reference to the ethmoid cell Anlagen are relatively far removed. The extensions into the conchæ, etc., would, therefore, in most cases be delayed—probably until puberty, or even later, when the ethmoid labyrinth reaches its full development. This explains Knight's statement: "Children seem to be exempt. None of my patients was under 20 years of age."

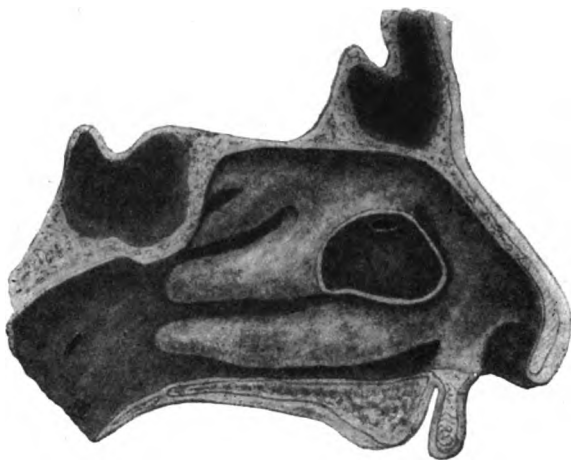


FIG. 6. Drawing from a specimen of the lateral nasal wall. Note the large cell in the concha media communicating with the infundibulum ethmoidale.



FIG. 7. Drawing of a frontal section through the head of an adult. Note the bilateral conchal cells in the middle nasal conchæ.



Figs. 4 and 5 show the extension of ethmoid cells into the *aggr nasi*. There is no reason why cells in this location should not extend farther, and finally reach and occupy the ventral end of the *concha inferior*. Such an extension would explain Schaeffer's cell of the ventral end of the inferior nasal concha.

Occasionally multiple cells are present, separated by thin partitions—each cell having an independent ostium, or in communication with either a cell of the anterior or one of the posterior group (fig. 5). Both *conchæ mediæ* of the same individual may contain cells (fig. 7), or one may be free of such cavities and the other contain one or more cells. Figs. 6 and 7 show the bullous type of cells—termed by Zuckerkandl "*conchæ bullosæ*."

Sex apparently does not have any bearing on the development of conchal cells, and they are about equally divided as to whether the ostia open superior or inferior to the attached border of the *concha media*.

The cells found in the *conchæ*, uncinæ processes, *aggr nasi*, and those extending into the frontal and sphenoidal sinuses, do not differ in any manner from the ethmoid cells of the lateral masses. The mucous membrane lining these conchal cells is extremely thin, but corresponds in its general structure with that lining the other ethmoid cells—unless changed by a pathological process.

Santorini thought the condition quite common, but says he should not venture to say that the condition is constant. He apparently regarded these cells as much more common than they really are. According to Reardon, Zuckerkandl observed them eight times in 172 skulls. Knight says that Zuckerkandl "found them thirty-six times in 200 post-mortem examinations." Lothrop found them in 9 per cent of all cases, and 11 per cent of the writer's specimens showed cells in the locations mentioned in a previous paragraph. The latter conclusions are based upon an examination of 150 adult nasal fossæ.

CONCLUSIONS

1. The cells found in the concha nasalis media, agger nasi, and processus uncinatus are true ethmoid cells, because:

(a) They differ in no manner from the cells of the lateral ethmoidal masses;

(b) Their ostia are invariably located at the points from which the ethmoid cells developed their Anlagen;

(c) They are frequently merely portions of lateral mass cells.

2. The cells invariably have ostia which communicate either with the superior meatus, with the ethmoidal infundibulum, or directly with the middle meatus.

3. The conchal cells may become the seat of a mucocele, abscess, etc., just as may the cells of the lateral ethmoidal masses, but to say that these cells owe their genesis to such pathological conditions is erroneous.

4. The so-called middle conchal sinus formed by the lateral and superior curling of the free border of the concha media is not homologous with, nor analogous to a conchal cell; nevertheless in some cases it may retain fluid in its hammock-like fold. The majority of conchæ mediæ, however, do not show this sinus, and when present it is, as a rule, of minor importance.

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## SUPPLEMENTARY ANNOUNCEMENT

### THE II. INTERNATIONAL CONGRESS OF ANATOMY

*Brussels, August 7-11, 1910*

The American Association of Anatomists is one of five national anatomical associations participating in the II. International Congress of Anatomy. This congress will constitute the twenty-sixth session of the American Association of Anatomy. Members intending to attend this congress are requested to notify the Secretary of the American Association of Anatomists at an early date and transmit to him titles of papers or demonstrations they desire to present. Titles must be in the hands of the Secretary of the American Association not later than June 25.

Inquiries concerning Anatomy, Comparative Anatomy and Embryology may be addressed to Professor Brachet, rue Snessens 18, or Doctor E. Willems, rue Paul Lauters 5.

Inquiries concerning Histology may be addressed to Professor Joris, rue du Président, or Doctor Sand, rue des Minimes.

Inquiries concerning lodgings and requests to have rooms reserved should be addressed to Doctor Brunin, Chef des travaux, 18 Avenue de la Renaissance.

The scientific sessions will be held during the forenoons, from 9 to 1 o'clock, in the physical lecture room of the University, 14 rue des Sols; the demonstrations in the Anatomical or Physiological Institutes, Park Leopold.

G. CARL HUBER, *Secretary*,  
1330 Hill Street, Ann Harbor, Michigan.

We are also informed by the Rockefeller Institute that Professor Jacques Loeb has been appointed head of the new department of experimental biology. "Here he will go on with his scientific work, following his own genius without special reference to problems in medicine, to which ultimately everything refers." Loeb is well known to anatomists by his work on regeneration, teratology and experiments of fertilization, and his appointment by the Rockefeller Institute is therefore of signifi-

cance to anatomists. That this Institute is so foresighted in its plans is a source of gratification to those who are promoting scientific anatomy. The splendid example that the Rockefeller Institute is setting in medical research may be received as a hint to our medical schools that are striving so hard to move forward. Every great medical school should be a Rockefeller Institute, and it is clear that this is possible only by the appointment of great men as professors.

We are authorized to announce that Prof. H. D. Senior of Syracuse University has accepted the call to the chair of anatomy at New York University (Bellevue). With one exception, this appointment completes the reform of the anatomical departments in the twenty-five leading medical schools. In them the example set by the University of Pennsylvania, Harvard, the University of Michigan, Columbia and Johns Hopkins has been followed by filling the chairs of anatomy with professional anatomists, which we believe to be a marked step in advance.

# THE RELATION OF THE MYOTOMES TO THE VENTRO-LATERAL MUSCULATURE AND TO THE ANTERIOR LIMBS IN AMBLYSTOMA

WARREN H. LEWIS

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WITH EIGHT FIGURES

## THE NORMAL DEVELOPMENT

The formation of the myotomes begins at an early stage before the tail bud makes its appearance. At a stage, shown in fig. 1,<sup>1</sup> in which there is a small tail bud present, there are fifteen myotomes and the sixteenth is partly formed. At this stage the anterior myotomes have just separated off from the lateral mesoderm. The first myotome is irregular in shape and lies in close relation with the gill mass. The third, fourth and fifth myotomes are still attached to the pronephros. The ventral processes of the first three myotomes are just beginning. Fig. 2, shows a stage, about two days later. There are no signs of the arm bud at this time, either in the dissected specimens or in the cross sections. From now on, there is a rapid growth of the ventral processes of the myotomes. The ventral processes of the anterior three myotomes pass in front of the pronephros, while the fourth lies behind. These ventral processes avoid the region of the pronephros and future arm bud and gradually grow over the lateral surface of the embryo, the first one faster than the second, the second faster than the third, and so on, so that the first and second may be well advanced before the seventh and eighth appear.

At a somewhat later stage than that shown in fig. 2, we find that ventral to the pronephros the ventral processes of the third and the fourth myotomes unite to make this lateral sheet continuous.

<sup>1</sup> The figures are all from dissected specimens, which were fixed in corrosive-acetic solution.

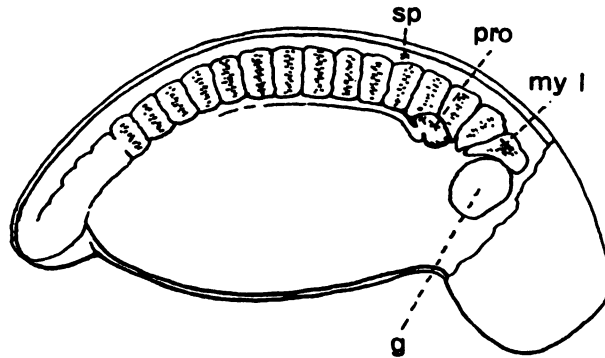


FIG. 1. Operating stage.  
my I, first myotome; pro, pronephros; sp, spinal cord; g, gill mass.

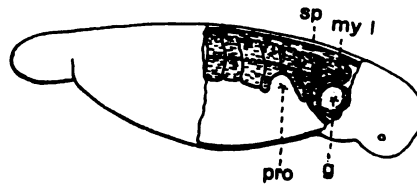


FIG. 2. Somewhat later stage showing ventral processes of anterior myotomes.

The ventral processes of the first, second, and third become completely separated from the myotomes, partly by the pronephros, and form the sterno-hyoid portion of the ventral musculature. From the first segment of the sterno-hyoid the genio-hyoid arises. The pronephros also gradually separates a portion of the ventral process of the fourth segment from its dorsal part. The segmentation of the ventro-lateral musculature can be observed even after the musculature is split into different layers. From the first and second myotomes a lateral chordal mass early splits off and is separated from the dorsal portion of the myotome by the vagus ganglion and the otic capsule. Fig. 3, shows normal relations of an embryo twenty days older than the one shown in fig. 1. The arm has been dissected away leaving only the myotomic musculature. The arm bud does not appear until sometime after the operation stage (fig. 1) and lies in close relation to the

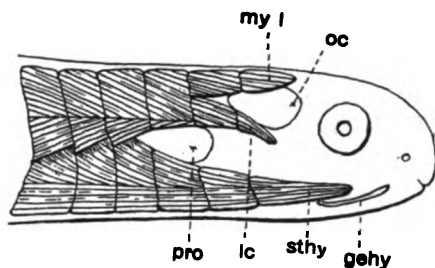


FIG. 3. Normal larva twenty days after operation stage, leg has been dissected away. *my I*, first myotome; *oc*, otic vesicle; *gehy*, genio-hyoid; *sthy*, stylohyoid; *lc*, lateral chordal muscle; *pro*, pronephros.

pronephros in the region of the second, third, fourth and fifth myotomes, mainly the third and fourth.

The cells of the arm bud apparently arise from the somatopleure in the region of the pronephros and not from the myotomes, they gradually form a protuberance on the surface of the embryo. The lateral myotomic muscle sheet gradually spreads out beneath the arm mass, that is medial to it but superficial to the pronephros.

#### EXPERIMENTS

In the following series of experiments with the exception of the second, I have attempted to remove various myotomes at the stage shown in fig. 1, that is, at the time just after the myotomes have separated off from the lateral mesoderm. The following results are based not only upon the study of dissected specimens but upon serial sections as well.

In the first series attempts to remove the first myotome only were not entirely successful, although in a number of experiments it was found that twenty days after the operation the dorsal part of the first myotome was almost completely absent and in such cases the anterior segment of the lateral chordal mass was very small. In all of these experiments however, the anterior end of the ventral muscle mass, namely the sterno-hyoid was present, though smaller than normal in one case. The failure to extirpate completely the first myotome was probably on account of the diffi-



culty of cutting out the ventral portion, which lies in such close relation with the gill mass, and which gives rise to the anterior end of the ventral musculature.

In the second series of experiments, an attempt was made to remove the ventral halves of the first three myotomes at a stage slightly older than that shown in fig. 1. In one partially successful experiment killed twenty days after the operation the dorsal part of these three myotomes was intact. The lateral chordal part of the first myotome was entirely wanting and that of the second myotome very much smaller than normal; the first segment of the ventral muscle mass, that is of the sterno-hyoid, was entirely wanting, and also the genio-hyoid which arises from it. The second ventral segment was very small and thin, while

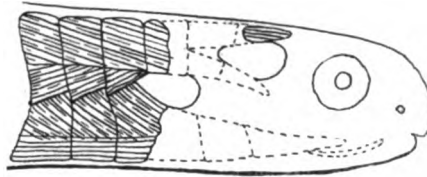


FIG. 4. Larva twenty days after removal of anterior three myotomes (first and third only partly removed). The lateral chordal and ventral derivatives of these myotomes are wanting.

the third was apparently normal. In the remaining experiments of this series the dorsal and lateral chordal portions of the myotomes are apparently uninjured while the sterno-hyoid and genio-hyoid muscles are smaller than normal especially at the anterior end where they are almost completely wanting in some of the experiments. It is evidently difficult to remove the ventral portion of the myotome entirely at this stage.

In the third series of experiments an attempt was made to take out completely the first three myotomes with the ectoderm over them. In an embryo killed twenty days after the operation, the conditions were found as shown in figure 4. The dorsal part of the first myotome is small, the first segment of lateral chordal mass and the sterno-hyoid are wanting as well as the genio-hyoid.

The second myotome as well as its derivatives is entirely absent. The third myotome is small and the third segment of the sterno-hyoid is absent. It is very evident that one need not extirpate the entire myotome in order to prevent the development of the ventro-lateral derivatives. In another experiment, the embryo was killed ten days after the operation, the dorsal part of the first myotome was found to be very small, while that of the second and third were wanting. The lateral chordal as well as the ventro-lateral musculature derived from these segments was found to be absent as in fig. 4. The fourth ventral segment was, however much more elongated than the one shown in fig. 4. The other experiments of this series show various degrees of extirpation of these myotomes, usually with the corresponding absence of their derivatives, namely the lateral chordal mass and the sterno-hyoid

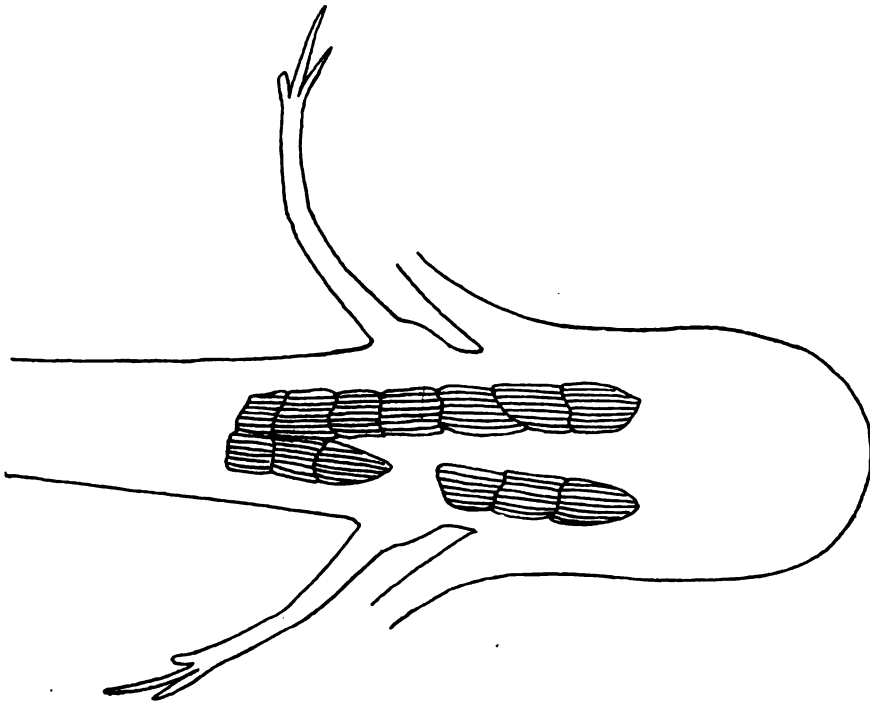


Fig. 5. Larva nineteen days after removal of the fourth myotome. Dorsal view.

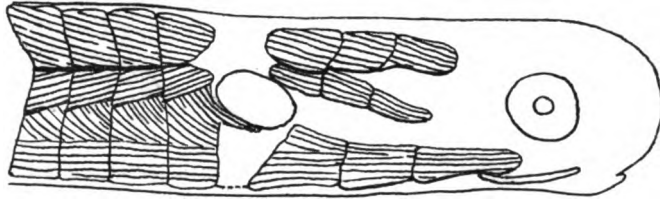
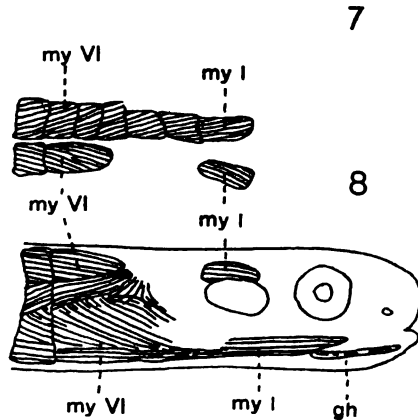


FIG. 6. Lateral view of larva shown in fig. 5. The ventral muscular derivative of the fourth myotome wanting.

and genio-hyoid muscles, or in some cases where these derivative muscles are not entirely wanting, they are found to be smaller than normal.

In the fourth series of experiments, I attempted to take out the fourth myotome only. In one very successful experiment, apparently this entire myotome was extirpated; as a result, we find nineteen days after the operation that the myotome and its muscle derivatives are entirely wanting, see fig. 5 and 6. In another experiment where the myotome was apparently entirely extirpated we find that the ventro-lateral muscle forms a continuous sheet. This is due to the elongation of the preceding and succeeding segments of the ventro-lateral musculature to fill in the gap.

In the fifth series of experiments, I attempted to remove the second, third, fourth and fifth myotomes. These myotomes were apparently completely extirpated in an embryo killed twenty-six days after the operation. Figs. 7 and 8 from a dissection show the following results. The second, third, fourth and fifth myotomes are completely wanting, as are also the muscular derivatives of these myotomes. We find that the sixth myotome (both dorsal and ventral portions) has elongated, the dorsal portion to nearly the length of three myotomes, and the ventral portion so as to partly fill in the gap between it (figs. 7 and 8) and the first segment of sterno-hyoid muscle. In fact, the ventral edge of the ventral muscle has extended so as to meet the elongated first segment. In another experiment, the third and fourth myotomes were apparently completely extirpated and the second and fifth



FIGS. 7 and 8. Larva killed twenty-six days after complete removal of the second, third, fourth and fifth myotomes. Dorsal and lateral views. The muscular derivatives of these myotomes wanting.

partially. The ventral lateral muscle shows complete absence of the third and fourth segments and imperfect development of the second and fifth, while the sixth segment is very much elongated. The gap, however, which might be expected from the complete absence of the third and fourth segments is nearly filled in by the elongation of the ventral portions of the remaining segments. Still other experiments show much the same results, the condition of the ventral musculature depending upon the degree of extirpation. This elongation of segments to fill in the gap caused by the extirpation of certain segments takes place in nearly all of the experiments and probably would be complete if the animals were allowed to live for a greater length of time after the experiment. The condition found by Miss Byrnes<sup>2</sup> in her experiments on *Amblystoma* in which, after destruction of the ventral halves of the myotomes in the region of the posterior limb the ventral musculature was present, is evidently to be explained through elongation of the remaining myotomes or their ventral processes. It is very unlikely that this regeneration takes place from the muscle of the opposite side as there is a wide gap between the two sides in the mid-ventral line.

## THE RELATION OF THESE EXPERIMENTS TO THE ANTERIOR LIMB

It was found in most of these experiments that extirpation of myotomes, either the first, second, and third, the fourth, or the second, third, fourth and fifth, made no difference in the development of the musculature of the leg, even complete absence of these myotomes was not accompanied by defects in the musculature of the limb. In a few experiments, however, the anterior limbs were absent or defective or the development was retarded, probably due to extirpation along with the myotomes of all or some of the cells destined to form the limb rudiment. These experiments show then very conclusively that the musculature of the limb is not derived from the myotomes. The experiments were primarily directed toward this problem and the extirpation of the myotomes was done immediately after and in some cases even before separation of the myotomes in the limb region from the lateral mesoderm and before there was any chance for myotome processes to have entered the place where the limb was later to arise. These results agree with those of Byrnes<sup>2</sup> on the relation of the limb muscles to the myotomes.

We have seen from fig. 2, that in the normal development the myotome processes avoid the region of the pronephros and of the limb, and that it has been impossible to trace in a study of the normal development either myotome buds or cells into the limb bud.

Both the experimental evidence and the study of the normal development support the idea that the musculature of the anterior limbs of *Amblystoma* develops *in situ* and is in no way derived from the myotomes or their ventral processes.

<sup>2</sup> *Journ. of Morph.* 1898. Vol. 14.

# LOCALIZATION AND REGENERATION IN THE NEURAL PLATE OF AMPHIBIAN EMBRYOS

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• WITH ELEVEN FIGURES

## EXPERIMENTS ON *RANA PALUSTRIS*<sup>1</sup>

It has already been shown that the dorsal and lateral lips of the blastopore of *Rana palustris*, when transplanted into slightly older embryos, possess considerable powers of self-differentiation into chorda, muscle, and nervous tissue. At this early stage, chorda and muscle differentiate much more completely and normally than nervous tissue.<sup>2</sup> Evidently the cells in the lips of the blastopore destined to form nervous tissue possess to a limited extent only the power of self-differentiation when thus transplanted and removed from their normal environment, while the cells destined to form chorda and muscle have already attained greater powers of self-differentiation.<sup>3</sup> It is of course impossible at this early stage to distinguish in the lips of the blastopore by the ordinary histological methods the cells which are to form muscle from those that are to form chorda or nervous tissue. These experiments however indicate very clearly that there are very profound differences in the cells themselves apart from any environmental differences.

<sup>1</sup> All the embryos experimented upon, both *Rana palustris* and *Amblystoma*, were killed in Zenker's fluid, cut into serial sections, 10 $\mu$ . in thickness, and stained in hæmatoxylin and congo red.

<sup>2</sup> Lewis, Experiments on the regeneration and differentiation of the central nervous system in Amphibian embryos. *Am. Jour. of Anat.*, vol. 5, 1906. Preliminary note before the Am. Ass. of Anatomists. Dec. 27, 1905.

<sup>3</sup> Lewis, Transplantation of the lips of the blastopore in *Rana palustris*, *Am. Jour. of Anat.*, vol. 7, 1907.

In another series of experiments on somewhat older gastrulæ of *Rana palustris*, small pieces were cut out anterior to the dorsal lip, from the region which later would form the anterior part of the neural plate. Such pieces when transplanted into the mesenchyme in the otic region of an older embryo continue to differentiate into nervous tissue, with nuclear and reticular zones irregularly arranged. Small irregular ventricular spaces are sometimes found and nerves are often given off into the surrounding mesenchyme.<sup>4</sup> Most of these embryos were killed twelve days after the operation, yet there is no indication of any degeneration of the transplanted tissue such as occurred in the nervous tissue in the preceding series. There has evidently been a considerable advance in the power of self-differentiation of this nervous tissue from that found in the lips of the blastopore of the earlier stage.

In still older gastrulæ of *Rana palustris*, just before closure of the blastopore when the neural plate is faintly outlined, small pieces of the neural plate were cut out and transplanted into older embryos. Piece 3, (see fig. 1), from the region of the medulla, extending from the primitive groove part way to the neural fold was transplanted in such a manner as to be entirely surrounded by mesenchyme. The embryo was killed ten days after the operation and the sections show imbedded in the mesenchyme ventral to the otic vesicle a quite normal shaped medulla. A section through it is very similar to one through the normal medulla, (see fig. 2). This small unilateral transplanted piece has developed into a perfectly bilateral structure with a large ventricle and thin roof. Even the arrangement of the nuclear and reticular zones is bilateral and shows remarkable similarity to the arrangement in the section of normal medulla seen in the same figure. The transplanted piece becomes smaller at either end and the ventricle is entirely closed. Had the piece remained in its original place in the neural plate it would probably have formed only a portion of one side of the medulla and have taken no part in the formation of the roof of the ventricle.

<sup>4</sup> Lewis, *Am. Jour. of Anat.*, vol. 6, 1907, p. 469, figures 5 and 6.

Piece 1, fig. 1, was transplanted in a similar manner and differentiated into what appears to be a somewhat irregular portion of the anterior end of the brain, with a small eye showing invagination and differentiation of the various layers of the retina. A nerve is given off from the caudal part of it. (figs. 3 and 4).

These two experiments alone are sufficient to indicate very clearly that the early neural plate of *Rana palustris* not only possesses great power of self-differentiation but that already there

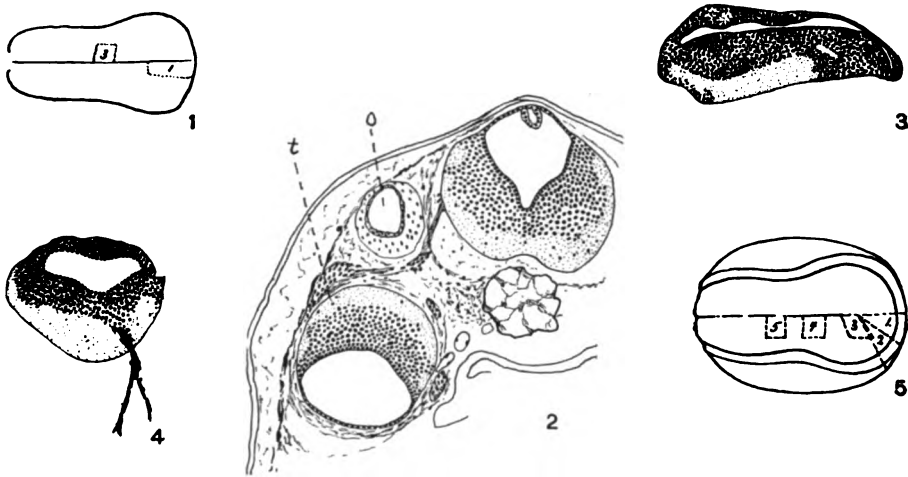


FIG. 1.—Outline neural plate *Rana*, pieces 1 and 3 transplanted.

FIG. 2.—Section showing transplanted piece 3, ten days after transplantation. t, transplanted piece; o, otic capsule.

FIGS. 3 and 4.—Sections through transplanted piece 1, thirteen days after transplantation.

FIG. 5.—Outline of neural plate *Amblystoma*, pieces 1, 2, 3, 4 and 5, transplanted.

is a localization in the various regions of cells or groups of cells that are destined to form certain parts of the central nervous system. The ordinary histological or microscopic examination of the neural plate does not reveal these differences yet the experiments show that a small piece from the region of the neural plate which one might expect from its location would form part of the medulla will do so whether it remains in the normal position or not.



Not only do these pieces differentiate into certain specific parts of the neural tube but they regenerate some of the surrounding parts such as the roof of the ventricle and in some cases the opposite side. In the case of piece 3, fig. 1, which developed into the bilateal medulla-like structure (fig. 3) the piece was unilateral and did not extend to the edge of the neural plate so it must

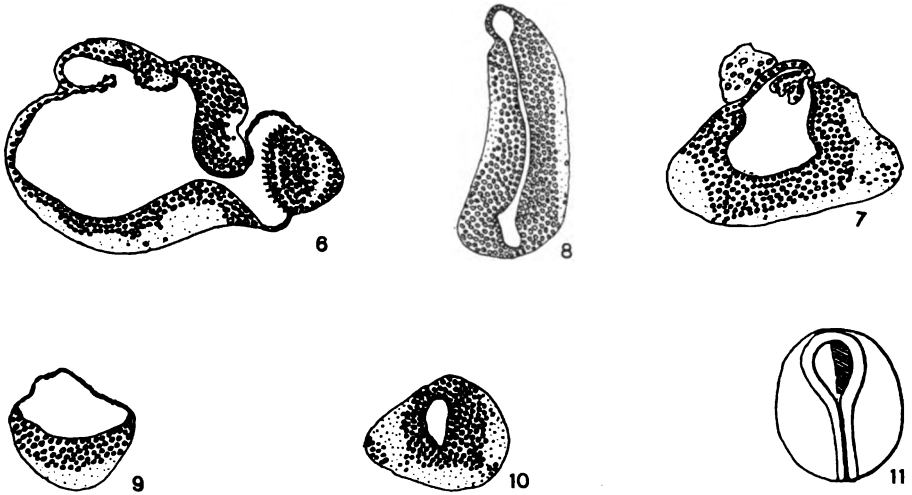


FIG. 6.—Section through transplanted piece 1, twenty-two days after transplantation.

FIG. 7.—Section through transplanted piece 2, twenty-five days after transplantation.

FIG. 8.—Section through transplanted piece 3, fifteen days after transplantation.

FIG. 9.—Section through transplanted piece 4, fifteen days after transplantation.

FIG. 10.—Section through transplanted piece 5, twenty-five days after transplantation.

FIG. 11.—Neural plate *Rana palustris* showing area cut away. Total regeneration followed.

have regenerated from itself the opposite half and the roof of the ventricle as well as some tissue on the same side. It has thus developed into a much more extensive piece than it would have, had it remained in the normal position. This would indicate that neighboring parts of the developing neural plate have under normal conditions a repressive influence on each other.

These transplanted pieces of the neural plate always close over to form a neural canal, the shape of the canal or ventricle varying with the region from which the piece is taken. The power of forming a neural canal then, resides within the neural plate itself or any portion of it and is not necessarily bound up with mechanical influences from other parts of the embryo. In like manner the longitudinal foldings of the brain, its flexures or bends, are probably due to intrinsic factors within the brain itself. The transplanted piece 1, shows indications of this process.

#### EXPERIMENTS ON THE NEURAL PLATE OF AMBLYSTOMA PUNCTATUM

The results obtained from many experiments on *Amblystoma* are similar to those from *Rana palustris*. As in the experiments on *Rana palustris* small pieces of the neural plate were cut out and transplanted into the otic region of somewhat older embryos. About one hundred and fifty such pieces were transplanted from the neural plates of twenty-seven different embryos. Two to eight pieces from each plate.

Pieces were taken from practically every region of the neural plate, and were allowed to develop in the transplanted position from ten to twenty-five days. At the time of transplantation there is no indication, other than general topographical position, of the different regions of the central nervous system. The histological picture of the arrangement and of the character of the cells is practically the same throughout the neural plate and only in later stages does the arrangement become characteristic for each portion of the central nervous system.

Fig. 5, for example, shows the position of five pieces which were cut out of the neural plate and transplanted into the otic region of an older embryo of *Amblystoma*. Figs. 6, 7, 8, 9 and 10, are from sections through these pieces—1, 2, 3, 4 and 5 respectively, which were allowed to develop for a number of days after the transplantation. Each piece has developed into a more or less characteristic form, corresponding somewhat to sections through the normal brain, medulla and upper part of the spinal cord. After the study of a number of such pieces and comparison

of them with sections through the normal central nervous system it was possible, without knowing beforehand from which region of the neural plate the piece was taken, to tell quite accurately its original location.

In normal embryos killed twelve days or more after the neural plate stage, transverse sections through the various regions of the brain and cord are very characteristic for each region, not only in the general outline and shape of the outer surface and ventricle but in the arrangement of the nuclear and reticular zones as well.

The transplanted pieces always close in to form the neural canal or ventricle. The form of the external surface of the canal (ventricle), and of the walls varying according to the region from which the piece was taken. Each piece seems to develop into that portion of the central nervous system into which it would have developed had it remained in the normal position. Not only does this take place but there is to a certain extent regeneration of the opposite side.

In the region of the medulla and spinal cord this regeneration sometimes results in a bilateral structure developing from a unilateral piece. All the transplanted pieces were unilateral at the time of transplantation.

Since each piece develops into a tube-like structure no matter what its orientation may be in its strange environment, the indication is very clear that this process is dependent only on changes which go on within the piece itself. We can correctly infer from this that the rolling in of the entire neural plate, to form the tubular central nervous system, is dependent only on changes which take place within the neural plate itself and is thus quite independent of influences from the rest of the embryo.

Again, since each piece appears to develop into that portion of the central nervous system into which it would have developed had it remained in its normal position, we must conclude that at this stage every part of the neural plate possesses the power of self-differentiation and is not dependent either upon influences of other portions of the embryo or of neighboring parts of the medullary plate itself for its differentiation. The neighboring regions, however, do influence each other in a way in regulating

the development by preventing such regions from that additional growth which they show after transplantation, such as regeneration of a portion of the opposite side or the formation of a roof to the ventricle, etc.

#### REGENERATION

The preceding experiments have shown that even a small piece of the neural plate possesses great regenerative power when cut out and transplanted into a strange environment. Likewise the neural plate itself has the power of regenerating small areas that have been removed.

One of my pupils, Mr. Dirge, removed a considerable portion of the floor at the anterior end of the neural plate on one side of the median line in *Rana palustris*. We were not aware at the time, of the power of regeneration and had hoped to remove permanently that portion of the brain which gave rise to some of the cranial nerves supplying the eye muscles of one side of the head. It was found however that even after as large a piece as is represented by the shaded area in figure 11 was removed regeneration is practically complete and so far as one can judge from serial sections the brain after two or three weeks is perfectly normal, bilateral and all the cranial nerves are present. This was repeated on a number of embryos of this stage with the same result. In some embryos however, when considerable degeneration and disintegration of the tissue about the wound followed the operation, there was often imperfect regeneration on this side of the brain. There is evidently then a limit to the power of regeneration of lost parts of the brain at this stage but within certain limits regeneration is complete.

In a number of my experiments on the transplantation of the optic vesicle a portion of the adjoining brain wall was transplanted with the optic vesicle. In the majority of these experiments the piece transplanted was not very large and regeneration of the lost part was complete. In a few, however, where larger pieces were transplanted with the optic vesicle the brain in the region

from which the piece was taken did not succeed in regenerating the lost part entirely and shows defect on that side. In all embryos, however, the ventricle becomes closed, the thickness of the defective wall varying more or less with the size of the piece removed.

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## THE STAINING OF FATS IN EPITHELIUM AND MUSCLE FIBERS<sup>1</sup>

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*From the Anatomical Laboratory,<sup>2</sup> University of Missouri*

If a section of kidney be examined fresh in aqueous humor the cells of the convoluted tubules will usually be seen to contain a large number of small, more or less refractive droplets. If the section be cleared a few minutes in dilute potassium hydroxide the droplets become much more sharply defined and a great many more may be distinguished. The same procedure will show a large number of droplets in the liver, striated muscle, and other tissues. The droplets in the muscle fibers are the interstitial granules described by Kölliker, Knoll, Schaffer, and others; those in the liver, kidney, etc. are not so well-known. Albrecht has called the droplets demonstrable in this way liposomes (Liposomen).<sup>3</sup> Because of the convenience of this term it seems advisable to adopt it, although these droplets do not always consist entirely of lipoids. Some of the droplets are strongly refractive; others are only faintly refractive, but there are all gradations between these two types. The strongly-refractive droplets are in most cases the ordinary fat droplets; the others consist in part at least of lipoid substances.

<sup>1</sup> Presented at the 25th Session of the Amer. Ass. Anat., Boston, December, 1909.

<sup>2</sup> The Department of Anatomy is coöperating with the Missouri Agricultural Experiment Station in the study of the process of fattening. The present paper is one of a series published in connection with this work.

<sup>3</sup> The term "liposome" will be used in this paper to mean those refractive droplets, visible in fresh tissue after the above mentioned treatment, which may be stained with Herxheimer's scarlet red. After a brief exposure in absolute alcohol the liposomes can no longer be shown by a fat stain.

It is the purpose of this communication to show that the liposomes may be readily stained in fresh tissues with Herxheimer's scarlet red, and to call attention to some differences among them in staining properties and susceptibility to fixing reagents. Osmic acid and the simple alcoholic solutions of scarlet red and sudan will stain many of the liposomes, sometimes all of them; but in many tissues crowded with these structures, the stains just mentioned give negative results, when the alkaline-alcoholic scarlet red (Herxheimer's stain) gives decidedly positive results. By the use of Herxheimer's stain on fresh tissues it is easy to study the nature and distribution of the liposomes and their variations under different nutritive conditions. These results were mentioned briefly in the Appendix of a previous paper (6, p. 435). At that time I had very unsatisfactory results with Herxheimer's stain, and thought that the alkaline-alcoholic solutions of scarlet red prepared by Bullard were much better; but I have learned since that Herxheimer's stain, when used properly, is usually as good.

The technique used for the demonstration of ordinary fats seldom stains all the liposomes in a tissue. To demonstrate the ordinary fats, it is usually recommended that the tissue be fixed first in formalin. Frozen sections are then to be cut and stained with osmic acid, sudan, or scarlet red. A fat stain extensively used at present is a saturated solution of scarlet red prepared by dissolving the dye in boiling 70 to 85 per cent alcohol (Fischer's method). In order to stain all the fatty droplets, two essential changes must be made in the above-mentioned technique. (1) The tissues must be stained fresh. No fixatives are to be used. (2) The alkaline-alcoholic scarlet red (Herxheimer's stain) must be used.

The tissues examined have been mainly those of the calf, cat, dog, rat and frog. Some of the voluntary muscles were examined in all cases. Usually the kidneys and heart and sometimes the liver were studied. Frozen sections of the fresh tissues were examined as follows: (a) unstained in aqueous humor, normal salt, 1 per cent acetic acid, 1 to 5 per cent potassium hydroxide; (b) after staining in 1 per cent osmic acid; (c) after

staining with simple alcoholic solutions of scarlet red, i.e., saturated solutions prepared by dissolving the dye in boiling 70 to 85 per cent alcohol; (d) after staining with alkaline-alcoholic solutions of scarlet red (Herxheimer's stain). Some of the sections were usually preserved in 55 to 70 per cent alcohol, 10 per cent formalin, potassium bichromate, etc. before staining, to determine the effect of the fixatives.

*Examination of fresh material.* If frozen sections of fresh striated muscle be examined in normal salt solution the interstitial granules are readily seen arranged in longitudinal rows between the fibrils. Rather more granules may be seen if the muscle be teased fresh in aqueous humor. Still more of the finer granules are visible if the tissue be cleared in dilute potassium hydroxide. The granules vary greatly in size. As a rule the larger ones are sharply refractive and the smaller ones weakly-refractive, but often this distinction does not hold. Droplets of ordinary neutral fat may be distinguished by their being more refractive than any others. 1 per cent acetic acid apparently does not dissolve the granules but it often causes the weakly-refractive ones to become indistinguishable from the sarcoplasm in which they lie; the more refractive granules are not affected. These interstitial granules are Albrecht's liposomes. The first accurate description of them was given by Kölliker (18).

Two types of muscle fibers may usually be distinguished—the so-called dark and light fibers. The dark fibers are crowded with liposomes; the light fibers usually contain only a few. A dark fiber may contain a comparatively few coarse droplets or a large number of small ones; the light fibers may contain a large number of small, very faintly refractive droplets. All possible intermediate forms between typical dark and light fibers may be seen in some muscles. The proportion of dark fibers to light fibers varies in different species and in the different muscles of the individual. It also depends to some extent upon the age and nutritive condition of the animal. Often two types of fibers can hardly be distinguished. The distribution of the dark and light fibers has been described by Grützner (11), Knoll (17), Schaffer (19), and others for a large number of animals. It is clear from the work of



these observers that the darkness or cloudiness of the fibers is due in part at least to the presence of the interstitial granules, but this is not the only factor involved, since the dark fibers still appear decidedly darker than the light ones even after the interstitial granules have been dissolved out or rendered invisible. The above-mentioned investigators did not recognize the fatty nature of the interstitial granules except in those cases where they may be stained with osmic acid, sudan and scarlet red not being known at that time. As far as I have observed the granules are all isotropic, but I have not studied this point extensively.

In the kidney, as in the muscles, some of the liposomes are visible in aqueous humor and normal salt solution. Dilute potassium hydroxide shows them most clearly. After treatment with 1 per cent acetic acid the faintly-refractive liposomes are no longer visible, but the strongly-refractive ones are unaffected. The strongly-refractive liposomes, as in muscle, are apparently the ordinary fat droplets and may be demonstrated by the ordinary technique, *i.e.*, by fixation in formalin and staining in osmic acid or simple alcoholic scarlet red. The liposomes occur mainly in the secretory portions of the renal tubules; they are not so numerous in the clear protoplasm of the collecting tubules. In some tubules in the section no liposomes at all can be demonstrated. The amount of ordinary fat in the kidney varies greatly. Of the animals examined, the cat shows the greatest amount of fat in this organ; the ox, the least. Both the less refractive liposomes and the more refractive (ordinary fat droplets) may be found in a cross section of the same renal tubule, though usually only one kind is present.

The liver often contains a large number of strongly-refractive liposomes (ordinary fat droplets) which replace or obscure the less refractive liposomes. Livers in which there is little or no ordinary fat are best adapted for the study of the less refractive droplets.

*Staining with osmic acid.* Altmann (4) has shown that osmic acid is reduced by oleic acid and triolein, but not by tripalmitin, tristearin, or their acids. This has been very generally accepted; but Starke (20) and a few others maintain that osmic acid will

blacken all the fat if the sections be kept in alcohol for some time after the osmic treatment. Starke found that out of 150 frogs (*Rana esculenta*) there were only two in which the fat in the liver was blackened directly by osmic acid; in all the others it became black only after the osmic-alcohol treatment. These results together with some tests with supposedly pure fats led him to the conclusion that palmatin and stearin are blackened by the osmic-alcohol method. He believes that oleic fats are blackened directly by osmic acid and that palmatin and stearin are colored yellowish or brown but become black if kept in alcohol for some time after the alcohol treatment.

Handwerck (12) agrees with Starke that the osmic-alcohol reduction takes place in the tissues but does not accept his explanation. According to Handwerck pure palmatin and stearin do not give the secondary reduction in alcohol; but if a slight trace of olein be added some blackening may be obtained. Osmic acid is a very delicate reagent for oleic fats.

Heidenhain (13), rejects the osmic-alcohol treatment on the ground that it blackens some structures that are not fat and fails to blacken some that undoubtedly are.

The extent to which osmic acid stains the liposomes depends probably upon their chemical composition. Sometimes (muscles of one adult rat, and one adult dog) it blackened every droplet that could be shown by any other method; in other instances however (muscles of two calves, one adult dog, five sucking pups; parts of the kidney in most animals, etc.) it gave no color at all to any of the liposomes, though they were easily stained in these cases with Herxheimer's solution. Sometimes (muscles of rat, kidney of cat, etc.) the simple osmic treatment may give the liposomes a brown color which changes to black if the sections be washed in water and kept 24 hours in 80 per cent alcohol (Starke's method). But the secondary treatment in 80 per cent alcohol may remove the color completely in a short time. This was found to be the case in the muscles of an emaciated cat, two sucking pups, and several rats. 80 per cent alcohol dissolves a great many of the less refractive liposomes after an exposure of a few hours. Even 60 per cent alcohol may produce the same result. In two

instances (one pup and one rat) the liposomes of the muscles were nearly all colored brown by osmic acid. The sections were then washed with water and put in 60 per cent alcohol. The droplets were nearly all decolorized after an exposure of thirty minutes in this solution. On the whole osmic acid is a useful reagent for the study of the liposomes. It often gives a brown color to droplets not stained at all by the simple alcoholic solutions of scarlet red and sudan.

In some instances (muscles, kidney, and liver of some rats) excellent results were obtained by fixation in Altman's fluid. The sections were washed 24 hours, dehydrated rapidly, cleared in cedar oil, and embedded in paraffin. Thin sections were cut and examined in cedar oil. This method has the advantage that thin sections may be had and the nuclei and boundaries of the cells may be seen much better than in frozen sections; but, as pointed out above, many liposomes cannot be stained at all with osmic acid especially those that are very faintly refractive.

As to the chemical composition of the droplets that may be shown with osmic acid, it is known that the unsaturated neutral fats are stained black; but the large number of liposomes which stain brown or grey cannot be identified with certainty.

*Simple alcoholic solutions of scarlet red.* Daddi recommended a saturated solution of sudan in 96 per cent alcohol, but alcohol of this strength was found to dissolve some of the fat, so that solutions in the weaker alcohols soon came to be preferred. The stains in use for a long time were saturated solutions of sudan or scarlet red prepared by dissolving the dye in cold 70 to 85 per cent alcohol. These stains are very weak.

A decided improvement was made by Fischer (10) who suggested dissolving the dye in boiling alcohol. This solution is considerably stronger than those made with cold alcohol and it seems to be extensively used at the present time. Traina (21) prepared a saturated solution of scarlet red in 70 per cent alcohol and kept it with excess of the dye in an oven at 40° C for two weeks before using. This seems to be of about the same strength as Fischer's solution. Scarlet red is usually to be preferred to sudan because of the brighter color it gives to fat droplets after relatively short exposure.

For staining ordinary fat droplets Fischer's and Traina's stains are usually satisfactory, but they seldom stain any of the faintly-refractive liposomes. In the muscles of some of the cats and dogs examined, they stained all the liposomes; but in a great many instances they stained only a very few of the liposomes or none of them at all. The muscle fibers of five sucking pups, two young calves, and several of the rats were full of coarse droplets that stained readily with Herxheimer's stain but were not colored at all by the simple alcoholic stains. Some liposomes which are browned by osmic acid are not colored at all by these stains, and on the other hand the simple alcoholic stains are said in some cases to stain liposomes that osmic acid does not affect.

The simple alcoholic solutions of scarlet red and sudan are also very variable in their actions. Considerably more fat is often shown if the staining dish is not too tightly closed. If a very small amount of evaporation is allowed the stain is somewhat more effective. Care was taken to exclude precipitates in these cases by comparison with sections stained by other methods and with unstained sections in normal salt and dilute potassium hydroxide.

*Alkaline-alcoholic scarlet red (Herxheimer's stain).* This solution may be prepared by dissolving two grams of sodium hydroxide in 100 cc. of 70 per cent alcohol. Scarlet red is then to be added to saturation. The solution should not be heated. Alkaline-alcohol dissolves considerably more of the dye than does ordinary alcohol. This solution is therefore much stronger, and it is more effective than any other fat stain. Herxheimer (15) claimed for his solution that it would stain more intensely than simple alcoholic scarlet red, but he does not state definitely that it will stain any droplets not stained by the latter. He however quotes Erdheim (9) as having found droplets in the thyroid which could be stained by the alkaline but not by the simple alcoholic solution. This statement of Erdheim's is the only one known to me in the literature in which it is claimed that Herxheimer's solution will stain droplets not shown by any other fat stain.

H. H. Bullard in some work done in the Anatomical Laboratory

at the University of Missouri, first showed clearly the great superiority of the alkaline solutions. His results have not yet been published.

One of the strongest objections made against Herxheimer's stain is its tendency to form precipitates. It is claimed by Fischer, Traina, and others that the stain is inferior to the simple alcoholic solutions on this account mainly. This objection is indeed well-founded in some instances. Freshly-prepared solutions (less than one day old) are often worthless on this account; and this tendency to form precipitates may persist in older solutions. But usually solutions a few days old give no trouble at all in this respect. A stain should not be used if it forms precipitates.

To determine whether any given stain forms precipitates, a section may be put in a hollow-ground slide with a few drops of the stain. A cover glass is then sealed over it quickly to prevent evaporation. One may then watch the droplets stain under the microscope. A precipitate may be detected in this way as soon as it begins to form. The character of the precipitate of any solution may be studied by allowing it to form on a section and then examining it under high magnification. The small dark granules of the precipitate may often be readily distinguished from the lighter colored liposomes, so that even under these conditions there is no confusion. But in every doubtful case the stained section should be compared closely with fresh sections mounted in normal salt solution or dilute potassium hydroxide.

After staining, the sections should be washed in 60 per cent alcohol about thirty seconds and then transferred at once to distilled water to wash out the alcohol. After a few minutes in distilled water they may be mounted in glycerin. If the alcohol is not washed out the sections are decolorized in a short time. The staining may be done in small tightly-stoppered vials.

Herxheimer's solution stains apparently all the droplets which may be demonstrated with dilute potassium hydroxide in a fresh tissue. It stains with varying degrees of intensity. The strongly-refractive droplets stain an intense red, the faintly-refractive droplets, a faint red; but there are all gradations between these

two types. Sometimes fine faintly-tinged droplets can be shown which are not visible in the potassium hydroxide preparations. The differences in intensity of staining among the liposomes may be due to the kind or the amount of the lipoid substances present, or both these factors may be involved.

*Staining with neutral red.* Albrecht (1) states that if tissues be kept in sterile vessels at 37° C for twenty-four hours, the liposomes will then stain with neutral red and show myelin figures in polarized light. Muscle must be kept in normal salt solution under otherwise similar conditions to give the same result. He holds that if muscle be treated as above described it will show longitudinal rows of droplets between the fibrils, which stain with neutral red and show myelin figures. These droplets are supposed to be identical with those seen in fresh tissues.

Albrecht (3) states also that if fresh muscle be mounted in 5 per cent potassium hydroxide the liposomes are shown very clearly, and if the tissue be kept in this solution a few hours they show myelin figures. Albrecht calls those droplets which show myelin figures only after being kept several hours at body temperature, postmortem myelin, to distinguish them from those which show myelin figures in the living tissue (intravital myelin).

In several rats, free-hand sections of the kidney were cut immediately after death and stained in a 1:10,000 neutral red solution at 37° C for about one hour. The convoluted tubules were shown full of coarse deeply-stained droplets. The size and arrangement of these droplets show that they cannot to any considerable extent correspond to the liposomes. In one rat kidney a number of tubules, which did not show any liposomes at all, were shown full of coarse droplets by neutral red. I have not succeeded in staining any granules in muscle with neutral red.

Several times I have repeated Albrecht's experiment of keeping the kidney (in this case that of a rat) in a sterile vessel at 37° C for twenty-four hours after removal from the body. A large number of droplets showing myelin figures are to be seen, but they do not seem to me to correspond at all to the liposomes seen in the fresh tissue. The numerous small liposomes seen in the fresh tissue have all disappeared.

*Effect of fixation.* To test the effect of fixatives frozen sections were cut and put directly into them. The effect may be more rapid by this method than that obtained by the fixation of a block of tissue.

*Ten per cent formalin.* A considerable part of the droplets in the muscle fibers of many adult cats and dogs are unaffected by an exposure of several months or more in this solution. The same is true for a great many droplets in the liver, kidney, and other organs. The droplets unaffected by this solution are usually strongly refractive and may usually be demonstrated by the ordinary fat stains. They are presumably droplets of ordinary neutral fat.

In other cases there is a gradual loss of the fat when the tissue is preserved in this solution. The gastrocnemius of a kitten, which contained a large amount of fat when examined fresh, gradually lost fat until at the end of a week only a few coarse droplets could be demonstrated.

The gastrocnemii of six sucking pups were also examined. When stained fresh in Herxheimer's stain, nearly every fiber showed several coarse droplets in a cross section. In four of the pups the droplets stained brown in osmic acid, but the color disappeared after a short time in alcohol or glycerin; 80 per cent scarlet red stained only a few droplets. In these four pups a great many droplets in the muscle fibers were lost after an exposure of only thirty minutes in formalin, and after twenty-four hours in this solution only a few droplets could be stained. In the other two pups the droplets stained readily with all the fat stains and were not affected by an exposure of ten days or longer in formalin.

The muscle fibers of two adult dogs, two young calves, and a number of rats were found to contain a large number of liposomes which were gradually rendered unstainable in formalin. Most of the droplets were lost to the stain after one to five days in the fixative. The liposomes of the muscles of an emaciated cat were affected in the same way.

The faintly-refractive liposomes of the kidney are nearly all lost after one day or less in formalin. The droplets that stain black with osmic acid and deep red with simple alcoholic scarlet

red are not much affected by any of the fixatives, but a few hours in formalin is usually sufficient to render the weakly-refractive droplets unstainable.

55 to 70 per cent alcohol acts upon the liposomes in about the same way and somewhat more rapidly than formalin. Eighty per cent alcohol dissolves the faintly-refractive liposomes very rapidly.

Potassium bichromate is decidedly less rapid in its action than either alcohol or formalin but ultimately produces about the same result. Ciaccio (7, 8) preserves tissues in a formalin-bichromate acetic mixture which he believes fixes the lecithin so that it is not removed by the ordinary fat solvents. The tissues are taken through xylol into paraffin so that the ordinary fat droplets are dissolved out. The sections are stained on the slide with simple alcoholic sudan. I have made only a few observations with this fixative. A few of the coarse liposomes are apparently fixed by this fluid and may be colored a light orange with simple alcoholic sudan after the sections have passed through the fat solvents. But I believe with Aschoff (5) that it is not proved that the droplets in question are really lecithin.

It will be apparent from the foregoing remarks that when a piece of muscle or kidney is fixed in formalin, alcohol, potassium bichromate, etc., some of the liposomes are usually removed or rendered invisible. The number of liposomes lost varies in different individuals as well as in different species. Pups of the same litter may contain entirely different fats. The effect of the fixative also varies with the age and nutritive condition of the animal and the length of time it acts upon the tissue. The action of the fixative in one tissue may be unappreciable for weeks, and in another nearly all the liposomes may be removed in a few minutes. The action of all the fixatives is particularly rapid on fine droplets that are difficult to stain, such as those of the heart. Probably the varying effect of the fixative is due to the varying chemical composition of the liposomes.

*Solubility.* The liposomes are all readily soluble in absolute alcohol and ether. Weaker alcohols dissolve the faintly-refractive liposomes with varying degrees of rapidity. The statement that



the liposomes are dissolved in these fluids means only that they can no longer be demonstrated with the fat stains.<sup>4</sup> It is probable that some of the liposomes are only in part composed of lipoids and that in these cases only the fatty portion of the liposomes has been removed. When fixed tissues are treated with 5 per cent potassium hydroxide no more liposomes are shown than can be stained with Herxheimer's solution. To determine whether the liposomes have been removed from a muscle fiber, longitudinal sections should be examined, since the masses of sarcoplasm seen in cross section between the fibrils may simulate them closely. Albrecht believed that many of the liposomes contained only an external lipoid layer, while the central core was of a non-fatty character.

*Relation of the liposomes to the nutritive condition of the animal.* Knoll, (16) in the heart muscle of pigeons starved six to nine days, found that the droplets visible in the fresh tissue were much fewer and that by far the majority of these were faintly refractive. The strongly-refractive droplets were greatly diminished by starvation. My observations corroborate Knoll's. Several rats which were kept on low rations until they had lost over twenty per cent of their body weight showed the muscle fibers free from liposomes. An adult cat which had lost about the same per cent of its body weight showed only faintly-refractive liposomes in the muscle fibers. The ordinary fat droplets in the kidney were not removed in these animals. Apparently the ordinary fat droplets are removed from the muscle fibers in the earlier stages of hunger, and the faintly-refractive droplets in the late stages. My observations are, however, not extensive enough yet to justify this as a general conclusion. The relation of the liposomes to the nutritive condition is a promising problem for future investigation.

<sup>4</sup> In a few instances some of the interstitial granules of the muscle fibers were shown by 5 per cent potassium hydroxide after having been exposed to absolute alcohol for forty-eight hours. They were not as large as before and could not be stained by any fat stain. It is therefore evident that these granules consisted only in part of lipoids and that the lipoid portion was dissolved by the alcohol.

## SUMMARY.

The protoplasm of renal cells, muscle fibers, etc., shows usually a large number of small more or less refractive droplets (liposomes) when examined in aqueous humor, or dilute potassium hydroxide.

These liposomes may all be stained with Herxheimer's scarlet red if fresh tissues are used.

All the liposomes disappear after a short exposure to absolute alcohol; and the weaker alcohols remove the faintly-refractive liposomes more or less rapidly.

Preservation of the tissues in formalin, alcohol, potassium bichromate, etc., may affect a large per cent of the liposomes so that they can no longer be stained. The effect of the fixative may be very pronounced in a few minutes, or it may require several days to produce noticeable changes.

The evidence cited in the preceding pages seems sufficient to prove that the liposomes consist wholly or in part of lipid substances.

The staining of the lipoids with Herxheimer's scarlet red is a simple and accurate method for the further study of their character and distribution and their relations to cell metabolism.

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# THE EFFECTS OF VARIOUS FIXATIVES ON THE BRAIN OF THE ALBINO RAT, WITH AN ACCOUNT OF A METHOD OF PREPARING THIS MATERIAL FOR A STUDY OF THE CELLS IN THE CORTEX

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WITH FIFTEEN FIGURES

While endeavoring to obtain preparations of the brain of the albino rat (*Mus norvegicus* var. *albus*) that would be suitable for a study of the cells in the cerebral cortex I have had occasion, this past year, to investigate the histological changes produced in this material by various methods of fixation and of imbedding: the results of this investigation are given in the present paper. There are but few observations regarding the histological action of different fixatives on brain tissue, and none of the recorded investigations dealing with the effects of various preservatives on the weight and volume of the brains of mammals have been accompanied by an account of the structural changes these preservatives produce.

According to the observations of Donaldson ('94), of Hrdlicka ('06), and of Fish ('93), the age and physical condition of an animal, the length of time it has been dead before the brain is put into the fixing fluid, the amount of fluid used and the temperature at which it acts, are all factors which tend to produce variations in the weight and volume of the brain. In all of the experiments on the brain of the albino rat which are recorded in the present paper an effort was made to eliminate as many as possible of the factors which might be supposed to influence the results. The animal selected for each experiment was one that was presumably in a healthy condition. It was killed either by ether or by illuminating gas

and then weighed and measured. The brain was taken out as soon as possible after the death of the animal and placed on absorbent cotton in 40 cc. of the fixing solution whose action was to be tested. Except in one case (rat no. 5), all fixation was done at room temperature which was about 20°C. The brains of adult individuals were taken for all of the experiments but two (rats nos. 20 and 21). The exact age of the animal used was not known in any case; but this factor could have had little, if any, influence on the results, as none of the rats could have been over a year old and the majority of them were much younger. The physical condition of the animals, therefore, is the uncontrolled factor which might have affected the results, and to it can doubtless be ascribed the variations in the results which were obtained when brains of different individuals were subjected to similar treatment.

After remaining in the fixing fluid a given length of time, each brain was drained for a moment on filter paper, to remove the superfluous liquid, and then carefully weighed in a closed weighing bottle. After passing through the various grades of alcohol required by the method of fixation employed, the brains were brought into 70 per cent alcohol, where they remained for forty-eight hours. They were then drained and weighed a second time in order to determine the loss in weight due to the replacement of the water in the brain by alcohol.

In all of the earlier experiments the brains were divided longitudinally after they had been weighed a second time, and each half of the brain was imbedded by a different method in order to ascertain what structural changes could be attributed to the process of imbedding when the same methods of fixation had been employed. It was soon found that methods of imbedding commonly used for neurological material, as well as for other tissues, produce marked alterations in the structure of the cells in the cerebral cortex. Imbedding in paraffine after clearing with either xylol, oil of cedar, bergamot oil, or chloroform, does not give satisfactory preparations of the rat's brain when the details of cell structure are wanted. Celloidin, since it can be used without heat, is a very excellent medium for imbedding brain tissue. There are, however, several disadvantages connected with the

use of celloidin as an imbedding medium, not the least of which is the difficulty of obtaining unbroken series of very thin sections. Equally good results were obtained when brains were imbedded in celloidin according to the methods advocated by Hardesty ('02) and by Lee ('05) as when the very long method devised by Miller ('03) was employed. After experiments had been made with a number of different methods it was finally decided that the most satisfactory results were obtained by double imbedding in celloidin and paraffine according to the method of Bödeker ('08). The details of this method are given in the second section of this paper.

For convenience in description, the data collected in the course of this study are given in six tables. In each of the first five tables the first column gives the index numbers of the rats whose brains were used, while the second column denotes the solutions used for fixation. The next two columns show the weight of each brain on its removal from the fixing solution, together with the percentage gain or loss in weight as a result of the action of the solution; the computed weight of the fresh brain being taken as the standard. The fifth column gives the weight of each brain after it had remained in 70 per cent. alcohol for forty-eight hours; and the last column shows the percentage gain or loss in weight as a result of the replacement of the water in the brain by alcohol. All of the data are brought together in table 6 which gives for each rat, in addition to what is shown in the first five tables, the sex, body weight, body length, the length of time the brain remained in the fixing solution, and also the weight of the fresh brain as computed from body length and body weight according to the method given by Donaldson ('08, '09), which is based on formulas devised by Hatai ('08, '09). [See page 233.]

With the few exceptions noted, all brains were imbedded in celloidin or in celloidin-paraffine. Sections were stained with thionin, except in the two cases (rats nos. 43 and 44), where this stain did not give satisfactory results. The illustrations are from drawings of the large pyramidal cells in the cerebral cortex taken from frontal sections at the level of the optic chiasma. As far as possible cells were selected for drawing which represented the

average condition of the large cortex cells, after the brains had been subjected to a given course of treatment. In the various tables a star (\*) is prefixed to the index number of each rat from whose brain cells were selected for illustration.

**A. THE EFFECTS OF VARIOUS FIXATIVES ON THE BRAIN OF  
THE ALBINO RAT**

At the present time formaldehyde is very generally used for the fixation and preservation of the brains of man and of the higher mammals. This substance, commonly employed in a 4 per cent. solution (10 per cent. formalin) produces but slight alterations in form or in color and gives a good consistency to the tissues, although it causes a marked increase in weight and in volume. Table 1 shows the various solutions containing formaldehyde that were used as fixatives of the brain of the albino rat and their effects on the brain weight.

TABLE 1<sup>1</sup>

RAT NO.	SOLUTIONS USED FOR FIXATION	WEIGHT OF BRAIN IN GRAMS ON REMOVAL FROM FIXING SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
*1	4% Formaldehyde.....	2.5750	+33	1.5706	-19
2	4% Formaldehyde.....	2.8200	+54	1.6436	-10
4	4% Formaldehyde.....	2.6778	+50	1.6577	-7
3	Formol-Müller (cold).....	2.2437	+21	1.5537	-16
5	Formol-Müller (warm).....	2.1880	+22	1.8711	+4
*27	Alcohol-formol.....	1.6392	-10	1.5147	-16
18	Zenker-formol.....	1.6040	-2	1.3297	-18
37	Marina's fluid.....	1.2219	-33	1.2913	-29
*38	Marina's fluid.....	1.2146	-35	1.2546	-33
41	Sublimate-formol.....	2.3315	+21	1.6565	-14
46	Sublimate-formol.....	2.0512	+17	1.3687	-22
49	Sublimate-formol-acetic.....	1.7687	-2	1.5003	-17
50	Sublimate-formol-acetic.....	1.8944	+8	1.5221	-13
*32	Graf's fluid (5% formalin).....	2.1520	+23	1.7421	-0
33	Graf's fluid (10% formalin).....	1.9283	+7	1.5994	-12
10	Bouin's picro-formol.....	1.7881	-00	1.4663	-18

<sup>1</sup> In this and in other tables, the percentages given are based on the computed fresh weight of the brain which is shown in table 6.

The brains of three rats (nos. 1,2,4) were fixed for forty-eight hours in a 4 per cent. aqueous solution of formaldehyde which had been made neutral with bicarbonate of soda as, according to Bayon ('05), a formaldehyde solution that has an acid reaction is not suitable for histological purposes. In all three cases there was a large initial gain in the weight of the brain which was followed by such a loss in weight after the brain had been brought into 70 per cent. alcohol that at the second weighing each brain weighed somewhat less than its computed fresh weight. The alteration produced in the brain weight of rats by aqueous formaldehyde solutions are similar to those which this fluid causes in the brains of man and of sheep, according to the investigations of Parker and Floyd ('95), of Flatau ('97), and of Hrdlicka('06).

On making a histological examination of the brains that were fixed in a 4 per cent. solution of formaldehyde, it was found that this substance does not have as injurious an effect on the structure of the cells as do other fixatives that produce much less alteration in the brain weight. One of the large cells from the cerebral cortex of the half of the brain of rat no. 1 which was imbedded in celloidin is shown in fig. 1. There is no apparent shrinkage of the cell body and the cytoplasm stains evenly and appears uniformly distributed. The nucleus, however, has suffered considerably from the action of the fixative, as it is decidedly larger than normal and its reticulum is poorly preserved and stains very faintly.

A cell from the portion of the brain of rat no. 1 which was imbedded in paraffine after being cleared in chloroform is shown in fig. 2. This cell plainly shows the injurious effects produced by this mode of imbedding. The cell body is considerably shrunk-en, while the nucleus is slightly contracted and very irregular in outline. The smaller cells of the cerebral cortex do not seem to be as adversely affected by the paraffine imbedding as do the larger cells, and most of them appear fully as well preserved as do similar cells in brains that have been imbedded in celloidin or in celloidin-paraffine.

Many investigators have stated that for histological purposes formaldehyde gives the best results when used in combination with other fixing reagents. Of the various formaldehyde mix-



tures that have been devised, the Formol-Müller solution of Orth ('92) has been most highly recommended by Juliusburger ('97), and others as an excellent fixative for the central nervous system. The brain of one rat (no. 3) was fixed for twenty hours in Formol-Müller solution, which was kept at room temperature (20° C.); the brain of another rat (no. 5) remained for three hours in this solution heated to about 35° C. As shown in table 1, each brain had gained about 21 per cent. in weight when it was removed from the solution; the subsequent loss in weight was, however, about 20 per cent. greater in the case of the brain which had been fixed in the cold solution than in that which had been fixed in the warm solution. When these brains were examined histologically the fixation of the cell structures was found to be no better in the one case than in the other. In both brains the large cells of the cerebral cortex appeared very similar to those in brains that had been fixed in 4 per cent. formaldehyde, as there was a slight swelling of the nucleus and a poor fixation of the nuclear contents. As a cell fixative for the brain of the rat, therefore, this fluid seems to have no advantage over the simple aqueous formaldehyde solution.

Parker and Floyd ('95) recommend a solution composed of 6 volumes of 95 per cent. alcohol and 4 volumes of a 2 per cent. solution of formalin as an excellent preservative for the brains of higher mammals. This solution was used as a fixative of the brain of rat no. 27. As the brain had decreased 10 per cent. in weight when removed from the fixing solution (table 1), it is evident that the addition of alcohol to formaldehyde prevents the swelling which is a characteristic action of aqueous formaldehyde solutions on brain tissue. As a cell fixative this fluid does not give satisfactory results. Although there is but little shrinkage of the cell body, the cytoplasm is invariably vacuolated in the vicinity of the nucleus, as shown in fig. 3, while the nucleus itself is somewhat irregular in outline and its contents are vaguely defined and stain faintly.

Zenker-formol was used as a fixative of the brain of rat no. 18. The brain lost but 2 per cent. in weight as a direct result of the fixation; the later shrinkage, after the brain had been brought

into 70 per cent. alcohol, being 18 per cent. The most marked histological effect of this fluid is on the cell nuclei. These structures always appear shrunken and irregular in outline, while their contents are very poorly preserved. Large cells of the cerebral cortex of the brain that was fixed by this method appear much as does the cell shown in fig. 2.

Marina ('97) recommends as a fixative for the central nervous system a solution made as follows:

Alcohol (96 per cent).....	100 ccm.
Formol.....	5 ccm.
Chromic acid.....	10 cgm.

When used on the brain of the rat (nos. 37 and 38) this fluid produces marked alteration in the brain weight and also in the cell structures. There is an initial loss of from 33 per cent. to 35 per cent. in the brain weight which is not materially altered by subsequent treatment of the brain with 70 per cent. alcohol. One of the cells from the cortex of the brain of rat no. 38 is shown in fig. 4. There is little apparent shrinkage of the cell body as a whole: the cytoplasm appears uniform, but it stains much more intensely than does the cytoplasm of cells in brains fixed with other formaldehyde solutions. On the cell nuclei this fluid had a very peculiar action. In the great majority of cases the nucleus appears swollen, and it has a very irregular outline with many indentations, as if the fixation had set up an unusual chemical reaction between the fluid contents of the nucleus and those of the cytoplasm. In some cases the nuclear reticulum seems to be entirely broken up so that the nuclear contents, save for the nucleolus, appears to be composed of small, rounded, deeply staining granules; in other cases, as shown in fig. 4, there are a few irregular clumps of nuclear substance scattered among the granules. Marina's fluid produced a much greater distortion of the nuclear structure in the cells of the cerebral cortex than resulted from the fixation with any of the other solutions that were used during the course of these experiments.

Ewing ('98) states that a saturated solution of corrosive sublimate in a 5 per cent. solution of formalin gives a superior fixation

of ganglion cells, bringing out the so-called chromatic network with great clearness. The brains of two rats (nos. 41 and 46) were fixed with this fluid. Each brain gained considerably in weight as a direct result of the fixation, the greater gain (21 per cent.) being made by the brain of rat no. 41 which had remained the shorter time (four hours) in the solution. Both brains later lost considerably in weight, the loss being greater in the brain (rat no. 46) which had remained for twenty hours in the solution. This fluid gives a very much better preservation of the cell structures than might, perhaps, be expected from its effects on the brain weight. Very few of the large cells in the cerebral cortex show any evidence of shrinkage, and the cytoplasm always appears uniform. The nuclear reticulum is fairly well preserved and it stains deeply; but the nucleus itself is usually slightly enlarged. The large cells in the cerebral cortex of the brains fixed by this solution appear very much like that shown in fig. 13.

One of the solutions recommended by Cox ('98) as a fixative for the spinal ganglion cells of the rabbit is made as follows:

Corrosive sublimate (saturated aq. solution) . . .	30 parts
Formalin . . . . .	10 parts
Glacial acetic acid . . . . .	5 parts

Comparatively slight alterations are produced in the brain weight as a direct result of fixation in this solution (table 1: rats nos. 49 and 50), although after subsequent treatment with 70 per cent. alcohol the brain loses from 13 to 17 per cent. of its computed fresh weight, depending on the length of time it has remained in the solution. As a cell fixative for the brain of the rat this fluid cannot be recommended. In all cases the nuclei of the large cells in the cerebral cortex are swollen, and the nuclear reticulum appears much like that shown in fig. 1.

The picro-formol solution of Graf ('97) certainly suffers from the omission of acetic acid, as Lee ('05: p. 77) has stated. This solution, made with 5 per cent. formalin, was used as a fixative of the brain of rat no. 32. The brain gained 23 per cent. in weight as a direct result of the fixation; but after remaining in 70 per cent alcohol for forty-eight hours it weighed practically its com-

puted fresh weight. A cell from the cortex of this brain is shown in fig. 5. The cell outline is regular and the cytoplasm appears uniform; the nucleus, however, is swollen and there is a very poor preservation of the nuclear contents.

The brain of rat no. 33 was fixed in Graf's fluid made with 10 per cent. formalin. This fluid produces a very different effect on the brain weight from that which results from fixation with Graf's solution which contains a smaller amount of formalin (table 1: rat no. 32). The initial increase in the brain weight is but 7 per cent., and the subsequent loss in weight, after the brain has been treated with 70 per cent. alcohol, is sufficiently large to make the final weight of the brain 12 per cent. less than the computed fresh weight. The stronger solution does not give as good a preservation of the cell structures in the cerebral cortex as does the solution that contains the 5 per cent. formalin, as there is a distinct shrinkage of the cell body in addition to an alteration of nuclear structure similar to that shown in fig. 5.

The picro-formol solution of Bouin ('97), which was used to fix the brain of rat no. 10, gave a much better preservation of the nerve cells in the cortex than did any of the other formaldehyde solutions that were tried, and it produced practically no alteration in the brain weight. The brain was imbedded in celloidin-paraffine and sections of it show an admirable preservation both of cell and of nuclear structure. A careful comparison between the cerebral cells in this brain and those in brains fixed in the solution of Ohlmacher ('97) shows that the latter solution gives a slightly better fixation of the nuclei than is obtained with Bouin's fluid. No further experiments were therefore made with Bouin's fluid which is doubtless as excellent a fixative for the central nervous system as it seems to be for many other kinds of materials.

Judging from the results obtained on the brain of the rat, solutions containing formaldehyde give, in general, a good fixation of the cell body, but they tend to produce a swelling of the nucleus which is usually accompanied by a poor preservation of the nuclear contents.

Before the introduction of formaldehyde as a fixing and hardening reagent, bichromate of potassium ( $K_2Cr_2O_7$ ), either in simple

aqueous solution or in combination with sodium sulphate as "Müller's fluid," was very generally employed for the fixation of mammalian brains. Donaldson ('94) studied the action of this preservative on the weight and volume of the brains of sheep. He found that, in general, the weight of a brain increases according to the number of days it is left in the solution; the gain being about 17 per cent. as a result of one day's action of a  $2\frac{1}{2}$  per cent. solution, increasing to a maximum of 38 per cent. after an immersion of two years in the fluid.

TABLE 2

RAT NO.	SOLUTIONS USED FOR FIXATION	WEIGHT OF BRAIN IN GRAMS ON REMOVAL FROM FIXING SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
22	$2\frac{1}{2}$ % $K_2Cr_2O_7$ .....	2.8445	+73	2.1409	+31
*23	$2\frac{1}{2}$ % $K_2Cr_2O_7$ .....	2.5594	+52	1.7518	+ 4
*24	$2\frac{1}{2}$ % $K_2Cr_2O_7$ + alcohol.....	2.5073	+40	1.8885	+ 6
25	$2\frac{1}{2}$ % $K_2Cr_2O_7$ + alcohol.....	2.8169	+50	2.1797	+16
*8	Zenker then Müller.....	1.8716	+ 3	1.6666	- 8
19	Zenker (20% acetic acid).....	1.7451	+ 5	1.3167	-21
*9	Dahlgren then Müller.....	1.9000	+ 3	1.7273	- 7
43	Tellyesniczky's fluid.....	1.9643	+ 6	1.6372	-12
44	Tellyesniczky's fluid.....	1.7981	+ 3	1.4906	-14
3	Formol-Müller (cold).....	2.2437	+21	1.5537	-16
5	Formol-Müller (warm).....	2.1880	+22	1.8711	+ 4
18	Zenker-formol.....	1.6040	- 2	1.3297	-18

In table 2 is shown the effects on the weight of the brain of the albino rat of various solutions containing bichromate of potassium.

The brains of two rats (nos. 22 and 23) were subjected to the action of a  $2\frac{1}{2}$  per cent. solution of bichromate of potassium for forty-eight hours. The gain in weight as a result of the absorption of the fluid by the brain tissue was enormous, being 73 per cent. in one case and 52 per cent. in the other. The brain of rat no. 22, which made the greater initial gain in weight, still weighed 31 per cent. more than its computed fresh weight after remaining

in 70 per cent. alcohol for two days; while the brain of rat no. 23 weighed 4 per cent. more than the computed fresh weight after a similar course of treatment. In spite of the fact that both brains were considerably swollen when they were imbedded in celloidin-paraffine, the tissues appeared greatly shrunken when prepared sections were examined under the microscope. In each brain the large cells of the cerebral cortex were greatly contracted and the cytoplasm much vacuolated, as shown in fig. 6. The nuclei of these cells were also distorted in shape and their membranes appeared abnormally thick. Only traces of a nuclear reticulum could be found even in the most favorable cases. The smaller cells of the cortex were also contracted and badly preserved. This solution had a more injurious effect on the cell structures in the cerebral cortex than did any of the other fixing solutions that were used during the course of these experiments.

Donaldson ('94) found that if the brains of sheep are put into a solution made by adding  $\frac{1}{4}$  volume of 95 per cent. alcohol to a  $2\frac{1}{2}$  per cent. solution of bichromate of potassium the initial increase in the brain weight is somewhat less than when the  $2\frac{1}{2}$  per cent. solution of bichromate of potassium alone is used. Thinking that this mixture might give better preparations of the brain of the rat than were obtained with the simple bichromate of potassium solution, I used it as a fixative for the brains of two rats (nos. 24 and 25). The effects of this fixative on the weight of the brains of rats are similar to those which are produced on the brains of sheep, as the increase in weight, although large, is less than that caused by the bichromate of potassium solution (table 2)). This mixture gives a better fixation of the cell structures in the cerebral cortex of the brain of the rat than does the aqueous solution of bichromate of potassium, but it is by no means a satisfactory fixative for histological purposes. The structure of one of the large cells in the cerebral cortex of the brain of rat no. 24 is shown in fig. 7. The cell body is somewhat shrunken and the nuclear outline is much distorted. In the nucleus of this cell, as in the nuclei of the great majority of the large cells in the cortex of brains fixed by a  $2\frac{1}{2}$  per cent. solution of bichromate of potassium, there is no indication of a nuclear reticulum, the greater part of the

chromatin being collected around the nucleolus. The cytoplasm of the cell is not vacuolated, but it appears denser in some places than in others.

At the present time Zenker's fluid is much used for fixing material for cytological study, and it seems to give very excellent preparations of many kinds of materials. The value of this fluid as a preservative of brain tissue was tested on the brain of rat no. 8. After remaining for six hours in this fluid the brain was put into Müller's solution for forty-eight hours in order that it might be properly hardened. As shown in table 2, the weight of the brain was increased but 3 per cent. as a result of the fixation, and the subsequent loss in weight was only about 8 per cent. When this brain was examined histologically it was found that the cells in the cerebral cortex had been poorly preserved in spite of the fact that the mode of fixation employed had produced but a slight alteration in the weight of the brain. The structure of one of the large cells in the cerebral cortex of this brain is shown in fig. 8. The cell outline is fairly regular, but the greater part of the cytoplasm is condensed around the nucleus. Although the nucleus has maintained its normal shape and size, the nuclear contents stain rather faintly and only traces of a nuclear reticulum can be found.

If the amount of acetic acid in Zenker's fluid is increased from 5 per cent. to 20 per cent. and the solution thus modified used as a fixative for the brain of the rat, there is an initial increase of about 5 per cent. in the weight of the brain, which is followed by a loss of about 20 per cent. in weight after the brain has been brought into 70 per cent. alcohol (table 2: rat no. 19). This fluid gives a better fixation of the cell structures in the cerebral cortex of the brain of the rat than does Zenker's fluid, but it is by no means a satisfactory fixative for brain tissue. After fixation with this fluid the large cells in the cerebral cortex appear much like that shown in fig. 3.

The brain of rat no. 9 was fixed in Dahlgren's ('97) fluid and then hardened in Müller's fluid. Cell structures are much better preserved by this mode of fixation than by the Zenker-Müller treatment, although practically the same alterations in brain

weight are produced in both cases (table 2). As shown in fig. 9, which is a drawing of one of the large cells in the cerebral cortex of the brain of rat no. 9, there is no contraction of the cell body and no vacuolization or condensation of the cytoplasm after this method of fixation. The deleterious effects of the fixation manifest themselves only in the nucleus of the cell. This body appears shrunken and irregular in outline, and the nuclear reticulum is not clearly defined.

After ascertaining that the fixatives commonly employed for cytological purposes produce various artefacts in the testis cells of the salamander, Tellyesniczky ('98) devised a theoretically good fixative made as follows:

Bichromate of potassium.....	3 grms.
Glacial acetic acid.....	5 cc.
Distilled water.....	100 cc.

The brains of rats nos. 43 and 44 were fixed in this fluid. The initial increase in the weight of the brains was comparatively slight, being 6 per cent. in one case and 3 per cent. in the other; both brains lost about the same amount (17 per cent.) after being treated with 70 per cent. alcohol. Thionin did not prove to be a satisfactory stain for this material; and, therefore, the sections were stained with Delafield's hæmatoxylin which brought out the nuclear reticulum with great distinctness but did not give particularly sharp outlines to the cell body. Tellyesniczky's fluid gives a fixation of the cells structures in the brain of the rat fully as good as that obtained with Bouin's picro-formol solution; neither solution, however, gives quite as fine a fixation or permits of as brilliant staining as does the solution of Ohlmacher ('97), whose action will be described in detail later on.

The alterations produced in the brain of the rat by fixation with fluids containing both formalin and bichromate of potassium have been already described (rats nos. 3, 5, 18). With the exception of Tellyesniczky's fluid, all of the various solutions containing bichromate of potassium that were used as fixatives of the brain of the rat gave a very inadequate fixation of the cell structures in the cerebral cortex. Owing, doubtless, to the fact that it penetrates



tissues very slowly, bichromate of potassium causes a contraction of the cell body and fails to preserve the nuclear structure.

Corrosive sublimate, either in concentrated water solution or combination with other fixing reagents, has been used successfully by various investigators as a preservative of the cell structures in the central nervous system of the vertebrates. von Lenhossék ('95) and Flemming ('96) recommend a concentrated solution of corrosive sublimate in water as a fixative for nervous tissue. This solution was used on the brains of rats nos. 39 and 40. The swelling of the brain as a result of the fixation was practically the same whether the brain remained for four hours (rat no. 39) or for twenty hours (rat no. 40) in the solution (table 3). After treatment with 70 per cent. alcohol, each brain was found to weigh about 23 per cent. less than its computed fresh weight. A much better fixation of the cells in the cerebral cortex is obtained when a brain is subjected to the action of the solution for twenty hours than when the fluid acts for only four hours. Entirely satisfactory preparations are not obtained in either case, however, as the cytoplasm of the cells is invariably vacuolated, much like that shown in fig. 6. The nuclei are very well preserved by this method of fixation, and details of structure appear with great clearness after the sections have been stained with thionin.

A saturated aqueous solution of corrosive sublimate, to which 5 per cent. of acetic acid had been added, was used as a fixative of the brain of rat no. 29. As shown in table 3, the brain increased but 5 per cent. in weight as a direct result of the action of the solution, and it subsequently lost about 20 per cent. of its computed fresh weight after being washed and passed through the lower grades of alcohol into 70 per cent. alcohol. Sublimate-acetic is a somewhat better fixative for the cell structures in the cortex of the brain of the rat than is the concentrated aqueous solution used on the brains of rats nos. 39 and 40, and it gives a remarkably good preservation of the nuclei, as is shown by an examination of fig. 12. The rest of the cell, however, is not preserved in an entirely satisfactory manner, as the cytoplasm is invariably vacuolated, although there is no evident shrinkage of the cell body as is the case in many of the cells in the brains of rats nos. 39 and 40.

A physiological salt solution saturated with corrosive sublimate was used as a fixative of the brains of rats nos. 42 and 45. There was an increase in the brain weight as a result of the fixation comparable to that produced in other brains preserved in solutions containing corrosive sublimate (table 3). The initial increase in the brain weight, however, was over twice as great (16 per cent.) when the brain remained in the solution for twenty hours (rat no. 45) as when the solution acted on the brain for only four hours (rat no. 42). The appearance of the cells in the cerebral cortex of brains fixed by this method is about like that shown in fig. 12. After a brain has remained twenty hours in this solution the nuclei of the large cerebral cortex cells appear slightly enlarged, and their contents stain less sharply than when a shorter time (four hours) has been employed for the fixation of the tissue.

The solution employed by Lang ('78) for the preservation of planarians has recently been used with apparently good results as a fixative of nervous tissues. For use on the central nervous system this solution, according to Ewing ('98), is made as follows:

Corrosive sublimate.....	5 grm.
Sodium chloride.....	6 "
Glacial acetic acid.....	5 cc.
Distilled water.....	100 cc.

The effects of this fluid on the weight of the brains of rats nos. 35 and 36 are shown in table 3. The initial increase in the weight of the brains was not very large, being 15 per cent. in the case of the brain which had remained in the solution for twenty hours (rat no. 35) and 10 per cent. when the solution acted for four hours only (rat no. 36). After being treated with 70 per cent. alcohol, these brains lost a comparatively small amount (table 3), yet the fixation of the cell structures in the cerebral cortex was not as good as that obtained by fixation with other corrosive sublimate solutions which produce a much greater alteration in the brain weight. One of the large cells from the cerebral cortex of the brain of rat no. 35 is shown in fig. 11. The nuclear reticulum is well preserved and stains very clearly; but in many cells the

nucleus itself is slightly swollen, although it retains its rounded form. The cell body is contracted and the greater part of the cytoplasm is condensed around the nucleus.

The sublimate-osmic-acetic mixture of Cox ('98), which was used as a fixative for the brains of rats nos. 47 and 48, produces a much greater increase in the brain weight if it is allowed to act for three days than if the brain is removed from the solution at the end of two days (table 3). The brain of rat no. 48 was the only one fixed in a solution containing corrosive sublimate that

TABLE 3

RAT NO.	SOLUTIONS USED FOR FIXATION	WEIGHT OF BRAIN IN GRAMS ON REMOVAL FROM FIXING SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
39	Saturated aqueous sol. $\text{HgCl}_2$ . .	2.0760	+ 8	1.4695	-23
40	Saturated aqueous sol. $\text{HgCl}_2$ . .	2.0229	+11	1.4087	-23
*29	Sublimate-acetic . . . . .	1.8604	+ 5	1.4414	-19
42	NaCl + sublimate . . . . .	1.9927	+ 7	1.3947	-25
45	NaCl + sublimate . . . . .	2.1549	+16	1.5074	-19
*35	Lang's fluid . . . . .	2.0670	+15	1.6794	- 7
36	Lang's fluid . . . . .	2.0429	+10	1.7970	- 3
47	Sublimate-osmic-acetic . . . . .	1.9917	+ 2	1.5483	-12
48	Sublimate-osmic-acetic . . . . .	2.1555	+22	1.8365	+ 4
41	Sublimate-formol . . . . .	2.3315	+21	1.6565	-14
49	Sublimate-formol-acetic . . . . .	1.7687	- 2	1.5003	-17
50	Sublimate-formol-acetic . . . . .	1.8944	+ 8	1.5221	-13

did not weigh less than its computed fresh weight after treatment with alcohol. Owing to the presence of osmic acid, this solution blackens the tissues considerably and sections must be bleached with hydrogen dioxide before they can be stained with thionin. The histological effects of this solution on the brain tissue is somewhat better than that obtained with any of the solutions of corrosive sublimate previously described. Very few of the large cells in the cerebral cortex show any signs of a contraction of the cell body or of a vacuolization of the cytoplasm; and the nuclei are well preserved in all cases. This solution does not give

a uniform fixation of the cell structures, however, and therefore it is not the best solution that can be selected for the preservation of brain tissue.

Other corrosive sublimate solutions used in the course of these experiments contained various amounts of formaldehyde, and their action on the weight of the brain of the rat as well as on the structure of the cells in the cerebral cortex have already been noted. All of the corrosive sublimate solutions that were used give a very good fixation of the nuclei in the large cells of the cerebral cortex; but they have a tendency to produce a vacuolization in the cytoplasm, and so do not give a fixation of the cell body at all comparable to that of the nucleus.

The effects of various corrosive sublimate solutions on the weight of the brain of the albino rat are shown in table 3.

For comparative purposes the brains of two rats (nos. 26 and 28) were fixed in alcohol, although this fluid is very little used at the present time for cytological work unless one is employing the technique used to bring out the so-called "Nissl substance" in the cytoplasm of the nerve cells. The effects of this mode of fixation on the weight of the brain of the albino rat are shown in table 4.

TABLE 4

RAT NO.	SOLUTIONS USED FOR FIXATION	WEIGHT OF BRAIN IN GRAMS ON REMOVAL FROM FIXING SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
*26	Alcohol (30%).....	1.7753	- 0	1.6201	- 9
28	Alcohol (95%).....	1.4418	-22	1.4611	-21
30	Carnoy's fluid.....	1.8192	- 2	1.4077	-24
31	Carnoy's fluid.....	1.7575	- 3	1.3042	-23
*34	Carnoy's fluid.....	1.7416	- 2	1.3110	-28

As shown in the above table, there is less initial loss in weight when the brain of a rat is fixed in weak alcohol than when strong alcohol is used. These results accord with those that Donaldson

obtained by preserving brains of sheep in alcohols of different strengths. Practically the same cytological changes are produced in the brain by fixation in alcohol, whether a strong or a weak solution has been employed. As shown in fig. 10, which is a drawing of one of the large cells in the cortex of the brain which was fixed in 30 per cent. alcohol, this mode of fixation causes a very slight shrinkage of the cell body as compared with that produced by a  $2\frac{1}{2}$  per cent solution of bichromate of potassium. The nucleus, however, is very greatly contracted and it is surrounded by a fluid vacuole. The cytoplasm appears uniformly distributed throughout the rest of the cell body although it stains more deeply in some regions than in others.

The solution most in vogue at the present time for the fixation of the cell structures in the central nervous system of the vertebrates is the chloroform-alcohol-acetic mixture devised by Carnoy ('87), which is known to many neurologists under the name of van Gehuchten's ('88) fluid. This solution was used as a fixative for the brains of three rats (nos. 30, 31, 34). Although acting on these brains for different lengths of time, the solution produced about the same alterations in the brain weight (table 4) and in the structure of the cells of the cerebral cortex in all three cases. The initial loss in the weight of a brain as a result of fixation by this solution is very slight, varying from 2 per cent. to 3 per cent. in different cases; subsequently the brain loses from 23 per cent. to 28 per cent. of its computed fresh weight when brought into 70 per cent. alcohol. The histological action of Carnoy's fluid on the cell structures in the cerebral cortex of the brain of the rat is shown in fig. 13. The cell has seemingly retained its normal size and shape and the cytoplasm appears uniformly distributed. The nucleus, however, is somewhat swollen; yet it has retained its rounded form, and the nuclear reticulum is well preserved and stains sharply. Carnoy's solution does not give quite as good a fixation of the cell structures in the cerebral cortex as can be obtained with other fluids, especially with the Ohlmacher solution described below.

Of all of the various fluids that were used as fixatives of the brain of the albino rat, the solution of Carnoy as modified by

Ohlmacher ('97) gave the best preparations for a study of the size and structure of the cells in the cerebral cortex. Table 5 shows the effects of this solution, acting for various lengths of time, on the weight of the brains of different individuals.

TABLE 5

RAT NO.	LENGTH OF TIME IN HOURS SOLUTION ACTED	WEIGHT OF BRAIN IN GRAMS ON REMOVAL FROM THE FIXING SOLUTION	PER CENT. LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. LOSS IN WEIGHT
11	6	1.8267	- 8	1.6248	-18
6	5	1.6100	-12	1.4471	-22
13	4	1.5787	-17	1.4498	-25
14	3	1.5458	-16	1.4633	-20
15	3	1.3978	-16	1.3099	-21
16	3	1.4590	-18	1.4000	-21
*17	3	1.6390	-11	1.4875	-20
7	2	1.7389	- 2	1.4099	-21
12	2	1.6924	-10	1.5748	-16
21	2	0.2489	-14	0.2011	-30
20	1	0.2523	-16	0.2074	-31

In this, as in other series of experiments, brains of various individuals reacted differently although subjected to the same course of treatment. These variations in the results can doubtless be attributed, in great part, to differences in the size of the brains and to the physical condition of the animals at the time that they were killed. There is no swelling of the brain after fixation in Ohlmacher's solution; on the contrary, there is a loss of about 15 per cent. in the weight of the brain of an adult rat as a direct result of the fixation, which is followed by a further loss of about 5 per cent. after the brain has been brought into 70 per cent. alcohol. The alterations produced in the brain weight are practically the same whether the brain remains for two or for six hours in the solution.

In order to ascertain whether Ohlmacher's solution would give as satisfactory preparations of the brains of young as of adult individuals, the brains of two rats (nos. 20 and 21), killed when

they were about forty-eight hours old, were fixed in this fluid. Each brain had lost about 15 per cent. in weight on removal from the solution. This loss in weight was subsequently increased to about 30 per cent. after the brains had been treated with 70 per cent. alcohol. The fact that the brains of young individuals lose more weight than do those of adults after fixation with Ohlmacher's solution is doubtless to be attributed, in part at least, to the differences in the percentage of water in the brain tissue of rats of different ages. The brain of a very young rat contains about 10 per cent. more water than does that of an adult animal (Donaldson), and the replacement of this larger amount of water by alcohol would necessarily produce a greater alteration in the brain weight.

In brains of young rats, as well as in those of adults, there is such a uniform shrinkage of the brain substance after fixation with Ohlmacher's solution that only very slight traces of it can be detected on examining prepared sections of brains that have been properly imbedded. That the method of imbedding that has been employed can produce marked alterations in the cell structures of tissues that have been well fixed is shown by a comparison of figs. 14 and 15. These drawings are of cells in the cerebral cortex of different halves of the same brain (rat no. 17) that were imbedded in different ways. When a brain that has been fixed in Ohlmacher's solution is imbedded in paraffine after being cleared with chloroform or with any of the other substances commonly used for this purpose, there is invariably a shrinkage of the cell body, as shown in fig. 14, and a condensation or vacuolization of the cytoplasm. If, however, the brain is imbedded in celloidin or in celloidin-paraffine, the large cells in the cerebral cortex have the appearance of the cell shown in fig. 15. There is no shrinkage evident anywhere in the cell. The cell outlines are regular and the protoplasmic processes stand out with great clearness; the cytoplasm is uniform in appearance and evenly distributed throughout the cell. The nucleus always maintains its normal relations with the cell body and its contents are well preserved and stain very sharply.

According to Ohlmacher, this solution gives an adequate fixation of the human brain, subdivided by Meynert's section, in twenty-four hours. It seems probable, therefore, that this method of fixation would give satisfactory preparations of the brain of any mammal if allowed to act for the proper length of time. There seems to be no disadvantage whatever connected with the use of this solution as a fixative of brain tissue, unless it be the cost of the ingredients of which the solution is composed.

TABLE 6  
*Summary of Data Collected*

RAT NO.	SEX	BODY WEIGHT IN GRAMS		BODY LENGTH IN MM.	NORMAL WEIGHT OF FRESH BRAIN COMPUTED	SOLUTIONS USED FOR FIXATION	NO. HOURS SOLUTIONS ACTED	WEIGHT OF BRAIN IN GRAMS WHEN REMOVED FROM SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
1	♂	277	219	1.94	4% Formaldehyde.		48	2.5750	+33	1.5706	-19
2	♂	163	196	1.83	4% Formaldehyde.		48	2.8200	+54	1.6463	-10
3	♀	158	199	1.85	Formol-Müller (cold).....		20	2.2437	+21	1.5537	-16
4	♀	129	183	1.78	4% Formaldehyde.		48	2.6778	+50	1.6577	-7
5	♀	164	188	1.80	Formol-Müller (warm).....		3	2.1880	+22	1.8711	+4
6	♂	187	198	1.85	Ohlmacher.....		5	1.6100	-12	1.4471	-22
7	♀	137	184	1.78	Ohlmacher.....		2	1.7389	-2	1.4099	-21
8	♂	160	190	1.81	{ Zenker.....		6	1.8716	+3	1.6666	-8
					{ Müller.....		48				
9	♀	170	197	1.84	{ Dahlgren.....		4	1.9000	+3	1.7273	-7
					{ Müller.....		48				
10	♂	182	186	1.79	Picro-formol.....		4	1.7881	-0	1.4663	-18
11	♂	275	228	1.98	Ohlmacher.....		6	1.8267	-8	1.6248	-18
12	♂	206	207	1.88	Ohlmacher.....		2	1.6924	-10	1.5748	-16
13	♂	228	210	1.90	Ohlmacher.....		4	1.5787	-17	1.4498	-25
14	♂	169	194	1.83	Ohlmacher....		3	1.5458	-16	1.4633	-20
15	♂	126	157	1.65	Ohlmacher.....		3	1.3978	-16	1.3099	-21
16	♂	158	181	1.77	Ohlmacher.....		3	1.4590	-18	1.4000	-21
17	♂	232	199	1.85	Ohlmacher.....		3	1.6390	-11	1.4875	-20
18	♀	111	154	1.63	Zenker-formol.....		1½	1.6040	-2	1.3297	-18
19	♀	106	159	1.66	Zenker (modified)...		1½	1.7451	+5	1.3167	-21
20	♂	6		0.30	Ohlmacher.....		1	0.2523	-16	0.2074	-31



TABLE 6—Continued

RAT NO.	SEX	BODY WEIGHT IN GRAMS	BODY LENGTH IN MM.	NORMAL WEIGHT OF FRESH BRAIN COMPUTED	SOLUTIONS USED FOR FIXATION	NO. HOURS SOLUTIONS ACTED	WEIGHT OF BRAIN IN GRAMS WHEN REMOVED FROM SOLUTION	PER CENT. GAIN OR LOSS IN WEIGHT	WEIGHT OF BRAIN IN GRAMS AFTER REMAINING IN 70% ALCOHOL FOR 48 HOURS	PER CENT. GAIN OR LOSS IN WEIGHT
21	♀	6		0.29	Ohlmacher.....	2	0.2489	-14	0.2011	-30
22	♂	108	156	1.64	2½% K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> .....	48	2.8445	+73	2.1409	+31
23	♂	88	163	1.68	2½% K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> .....	48	2.5594	+52	1.7518	+4
24	♂	162	187	1.79	Alcohol K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> ...	48	2.5073	+40	1.8885	+6
25	♂	190	207	1.88	Alcohol K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> ...	48	2.8169	+50	2.1797	+16
26	♂	174	184	1.78	Weak alcohol.....	27	1.7753	-00	1.6201	-9
27	♂	168	191	1.81	Alcohol-formol.....	24	1.6392	-10	1.5147	-16
28	♂	221	198	1.85	95% Alcohol.....	24	1.4418	-22	1.4611	-21
29	♂	151	184	1.78	Sublimate-acetic....	1½	1.8604	+5	1.4484	-19
30	♂	213	202	1.86	Carnoy's fluid.....	3	1.8192	+2	1.4077	-24
31	♂	181	194	1.82	Carnoy's fluid.....	4	1.7575	-3	1.3042	-23
32	♀	141	178	1.75	Graf (5% formalin)	2½	2.1520	+23	1.7421	-00
33	♂	165	191	1.81	Graf (10% formalin)	1½	1.9283	+7	1.5994	-12
34	♀	149	184	1.77	Carnoy's fluid.....	19	1.7416	-2	1.3110	-28
35	♀	167	189	1.80	Lang's fluid.....	20	2.0670	+15	1.6794	-7
36	♂	208	203	1.86	Lang's fluid.....	4	2.0429	+10	1.7970	-3
37	♀	173	194	1.82	Marina's fluid.....	72	1.2219	-33	1.2913	-29
38	♂	197	201	1.86	Marina's fluid.....	96	1.2146	-35	1.2546	-33
39	♂	259	214	1.92	Cor. sublimate.....	4	2.0760	+8	1.4695	-23
40	♂	177	195	1.83	Cor. sublimate.....	20	2.0229	+11	1.4087	-23
41	♂	265	216	1.92	Sublimate-formol..	4	2.3315	+21	1.6565	-14
42	♂	213	203	1.86	NaCl + sublimate..	4	1.9927	+7	1.3947	-25
43	♀	213	204	1.86	Tellyesniczky.....	48	1.9643	+6	1.6372	-12
44	♀	137	177	1.74	Tellyesniczky.....	24	1.7981	+3	1.4906	-14
45	♂	196	200	1.85	NaCl + sublimate..	20	2.1549	+16	1.5074	-19
46	♀	135	179	1.75	Sublimate-formol..	20	2.0512	+17	1.3687	-22
47	♂	141	179	1.75	Cox (osmic).....	48	1.9917	+2	1.5483	-12
48	♂	150	182	1.76	Cox (osmic).....	72	2.1555	+22	1.8365	+4
49	♂	171	192	1.81	Cox (formol-acetic)	48	1.7687	-2	1.5003	-17
50	♂	137	178	1.75	Cox (formol-acetic)	72	1.8944	+8	1.5221	-13

B. A METHOD OF PREPARING THE BRAIN OF THE ALBINO RAT  
FOR A STUDY OF THE CELLS IN THE CEREBRAL CORTEX

Experience has shown that considerable time is often consumed in adapting a general method of preparation to the particular material with which one is working, and that in many cases comparatively slight variations in the lengths of time different fluids act on the tissues produce marked structural effects. For these reasons it has been thought advisable to give in detail a method of preparing the brain of the rat which produces satisfactory preparations for a study of the cells in the cerebral cortex, although in this method there is very little that is new. This method should give equally good preparations of the brain of any other small mammal, and it would doubtless be applicable also to small pieces of the brain of any of the larger animals.

For fixation the solution devised by Ohlmacher ('97) is used. This solution is made as follows:

Absolute alcohol.....	80 parts
Chloroform.....	15 parts
Glacial acetic acid.....	5 parts
Corrosive sublimate to saturation (about 20 per cent.)	

As the corrosive sublimate dissolves rather slowly, it is necessary to make up the solution a few days before it is required for use.

Brains of adult rats are well fixed after being subjected to the action of this solution for three hours: for the fixation of the brains of very young individuals an immersion of two hours in the liquid is sufficient. On removal from the solution the brain is placed in 85 per cent. alcohol, where it remains for about one hour. It is then transferred into iodized 70 per cent. alcohol, where it is kept until the corrosive sublimate has been extracted from the tissues. This latter process requires at least twenty-four hours, and if the brain has not been subdivided it is necessary to renew the liquid and keep the brain in it for two or three days. The brain is then brought into 80 per cent alcohol where it can remain as long as necessary. It is advisable to imbed the material as soon as pos-

sible, since long immersion in alcohol is injurious to any tissue and greatly lessens its staining powers.

For imbedding the celloidin-paraffine method of Bödeker ('08) gives quite as satisfactory preparations of brain tissue as does celloidin, and it has the great advantage of imbedding this material so that it can readily be cut in very thin serial sections, which can subsequently be treated as if paraffine alone had been the imbedding medium. The directions for this method as given by Bödeker are rather general, and the method as finally adapted to the brain of the rat is as follows: From 80 per cent. alcohol the brain is passed through 95 per cent. alcohol, absolute alcohol, and ether-alcohol, remaining in each solution for twenty-four hours. It is then transferred into 2 per cent. celloidin where it is left for two or for three days, depending on the size of the brain. After six hours immersion in chloroform the brain is put into benzole for one hour, and is then carried over into benzole saturated with soft paraffine where it remains eighteen hours. In order to facilitate the penetration of this solution into the brain tissue it is advisable to keep the liquid slightly warm (about 35°C.) The brain is then placed in melted soft paraffine (melting point about 45°C.), which is kept just above the melting point for the three hours that the brain remains in it. Subsequently the brain is brought into melted hard paraffine (melting point about 54°C.), which must be kept as near the melting point as possible since heat is very injurious to brain tissue. After remaining in the hard paraffine for two hours the brain is ready to imbed in hard paraffine. Brains thus prepared can be cut with a Minot microtome into serial sections which can be made as thin as 5 $\mu$  if desired.

The sections are mounted in the usual way with albumen fixative and the paraffine removed with xylol. In further treatment one must avoid the use of absolute alcohol, as this substance tends to loosen the sections from the slide. In place of absolute alcohol a mixture composed of equal parts of chloroform and of absolute alcohol can be used with safety. After passing through the various grades of alcohol into distilled water the mounted sections are stained for two or three minutes in a 1 per cent. solution of carbolic acid saturated with thionin. They are then washed for a

moment with distilled water and differentiated in 95 per cent. alcohol. The process of differentiation can be watched under a microscope, as it does not take place very rapidly. If a counter-stain is desired a small amount of eosin can be added to the alcohol in which the sections are differentiated. The slides are then passed quickly through the chloroform- alcohol mixture into xylol, and the sections are finally mounted in Canada balsam.

Although thionin is known to be an excellent stain for cytological purposes, it is little used when preparations are to be kept for any length of time, as it fades rapidly if used in an aqueous solution. As a stain for the cell structures in the cerebral cortex of the brain of the rat, thionin has been found to act more energetically and to give somewhat sharper outlines when dissolved in a weak solution of carboic acid than when used in an aqueous solution. In order to test the permanency of the stain, prepared slides were exposed for three months on a well lighted laboratory table. At the end of this time the sections were somewhat faded, but structural details could still readily be made out. Other slides similarly stained have been kept for over a year in slide boxes and the sections do not appear to have faded in the slightest degree. If the sections are not exposed to the light unnecessarily, it is probable that the stain will be as permanent as that given by the great majority of the anilin dyes.

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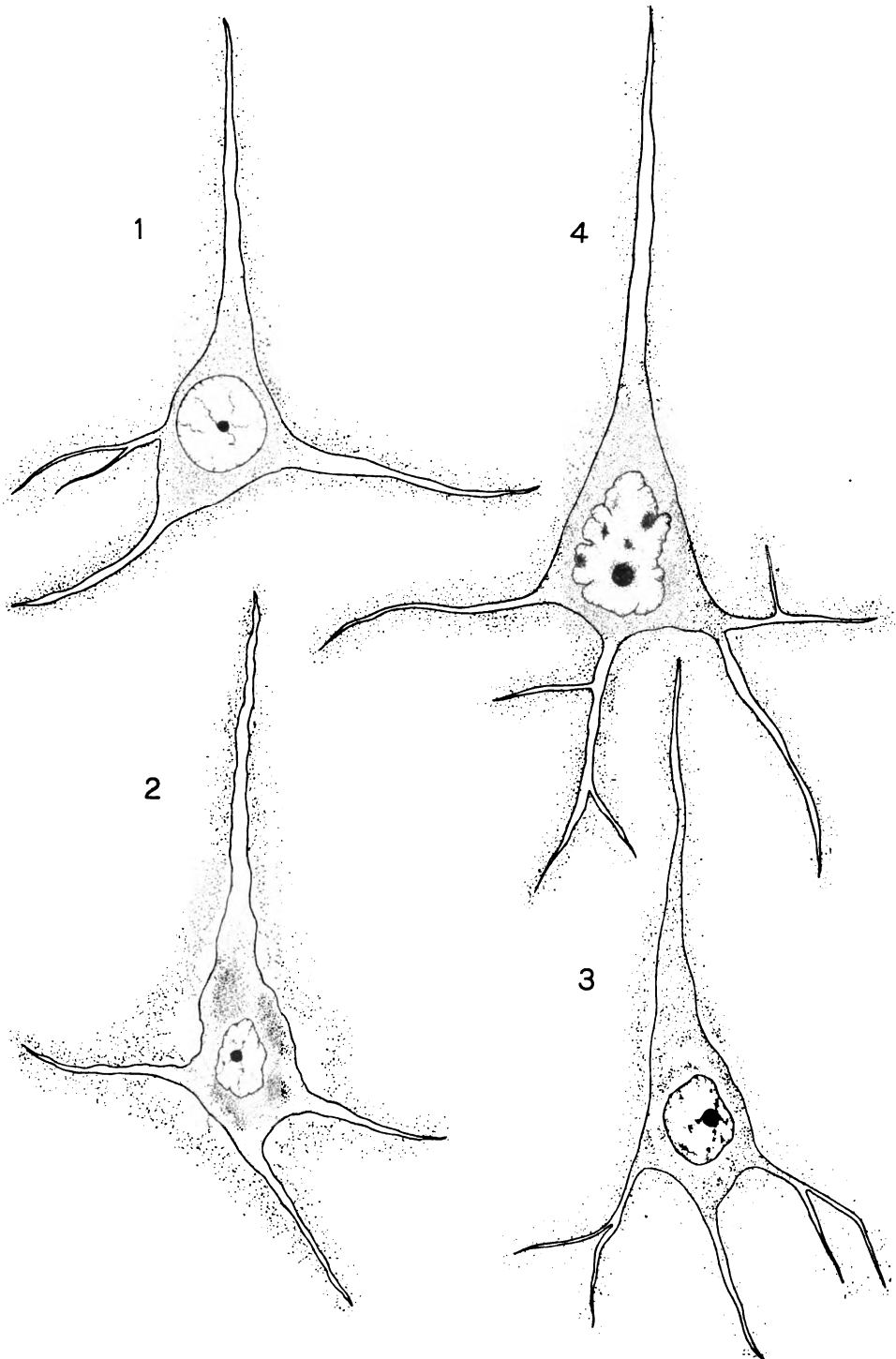
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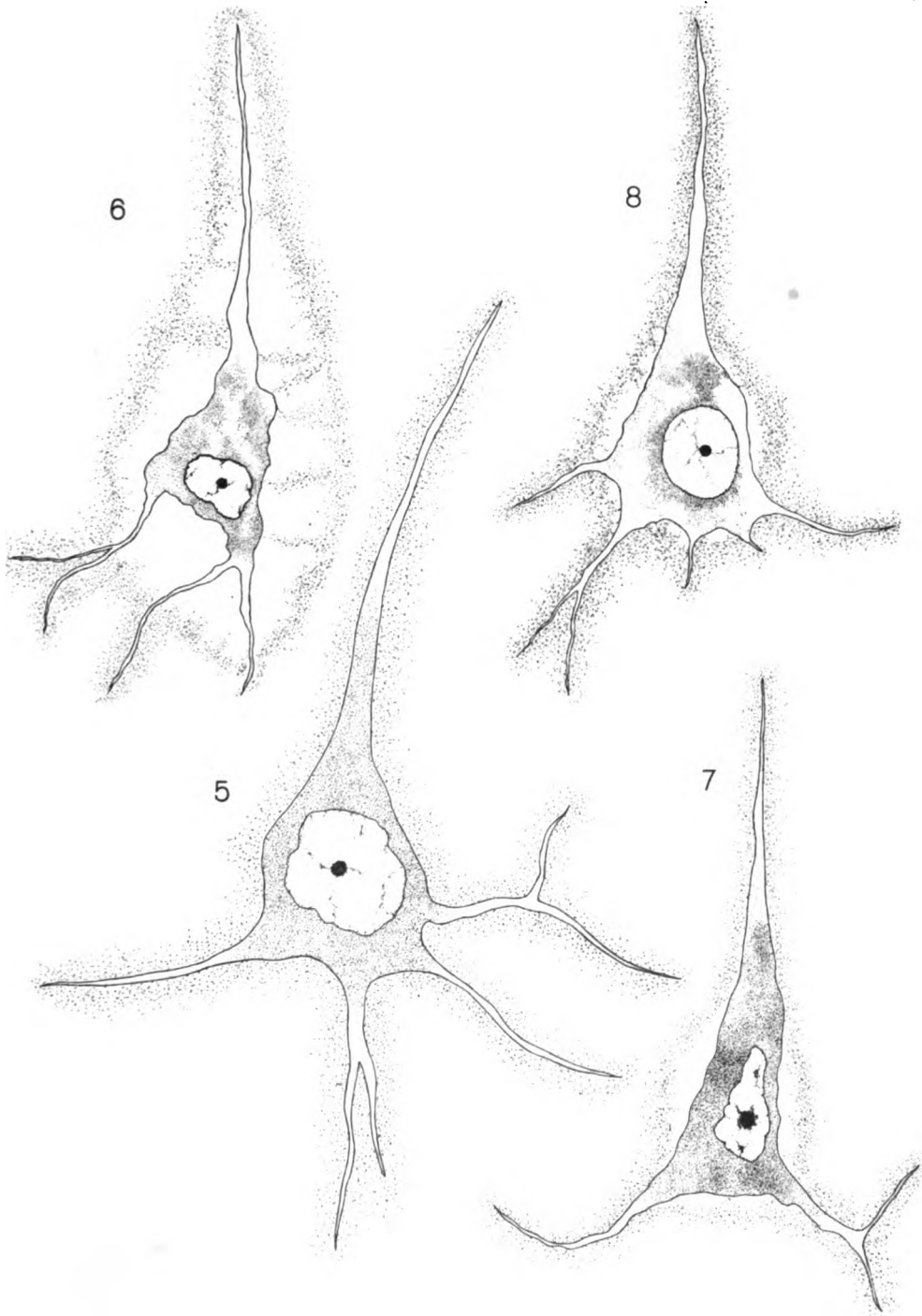
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## EXPLANATION OF FIGURES

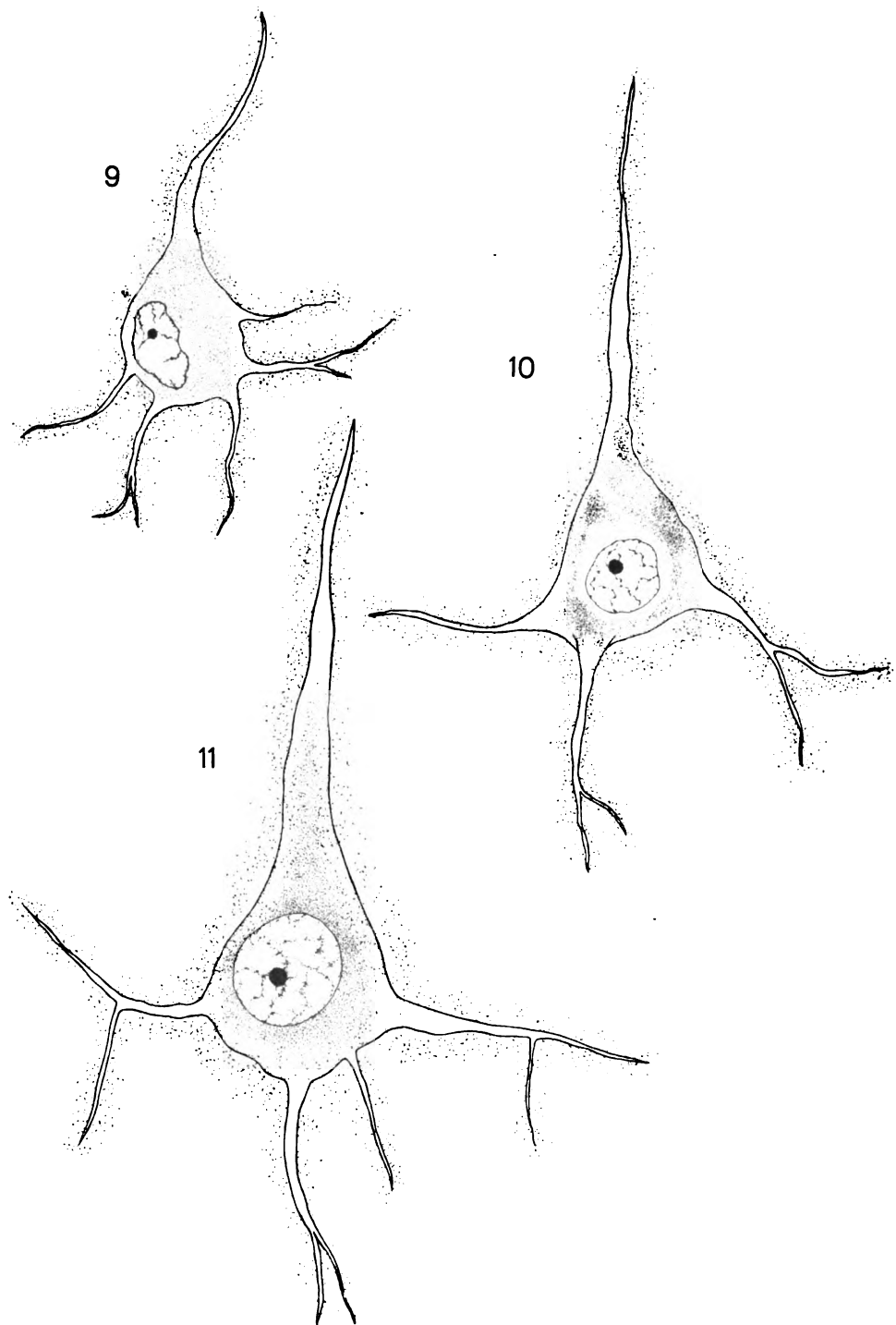
All figures were drawn with the aid of a camera lucida under a Zeiss apoc. 1.5 mm obj.; oc. 8. They have been reduced to give a magnification of about 800 diameters.

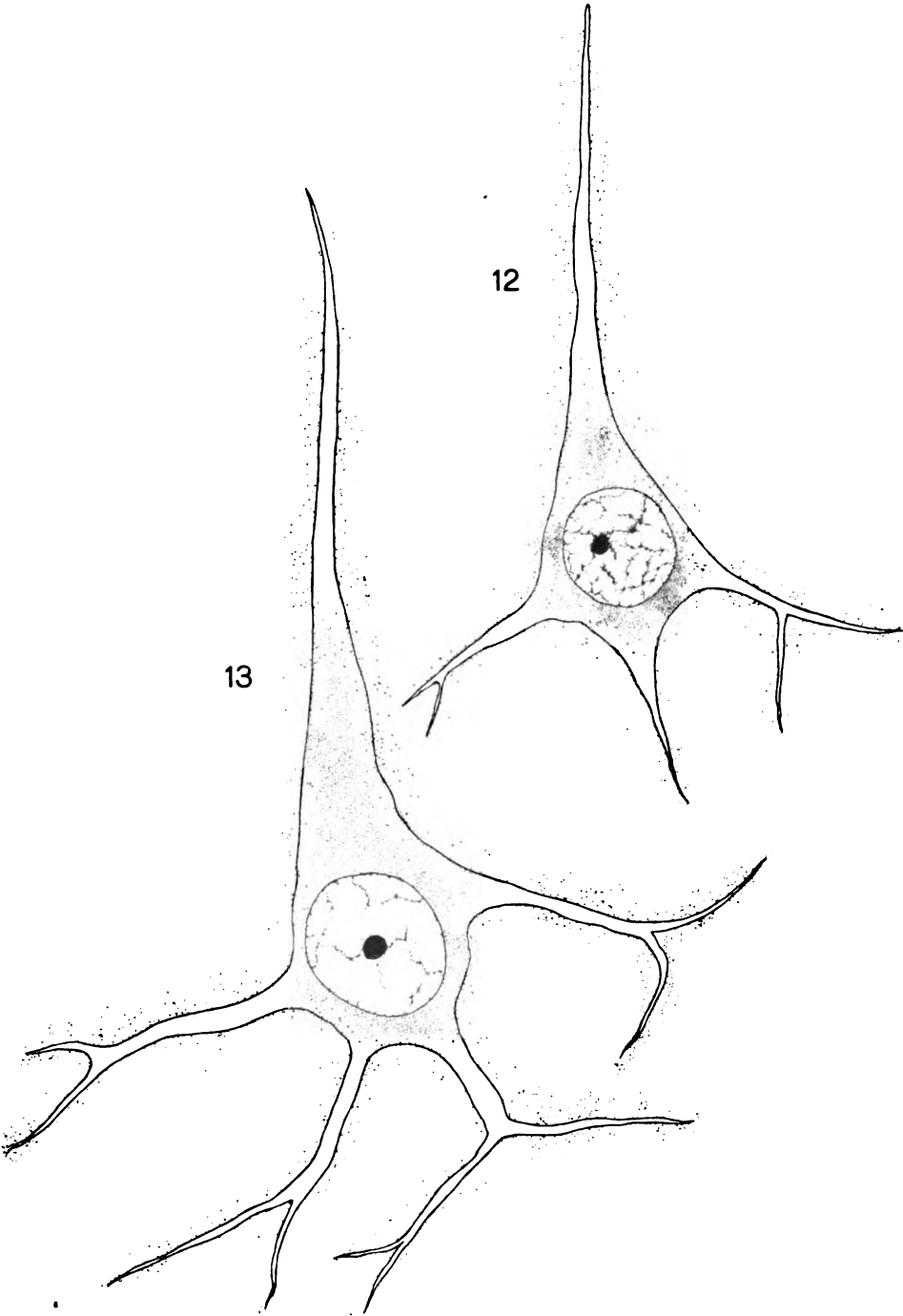
1. Cell from the cerebral cortex of a brain (rat no. 1) imbedded in celloidin after fixation in 4 per cent formaldehyde.
2. Cell from the cerebral cortex of a brain (rat no. 1) imbedded in paraffine after fixation in 4 per cent formaldehyde.
3. Cell from the cerebral cortex of a brain (rat no. 27) imbedded in celloidin-paraffine after fixation in alcohol-formol.
4. Cell from the cerebral cortex of a brain (rat no. 38) imbedded in celloidin-paraffine after fixation in Marina's fluid.
5. Cell from the cerebral cortex of a brain (rat no. 32) imbedded in celloidin-paraffine after fixation in the picro-formol solution of Graf.
6. Cell from the cerebral cortex of a brain (rat no. 23) imbedded in celloidin-paraffine after fixation in a 2½ per cent solution of bichromate of potassium.
7. Cell from the cerebral cortex of a brain (rat no. 24) imbedded in celloidin-paraffine after fixation in alcohol-bichromate of potassium.
8. Cell from the cerebral cortex of a brain (rat no. 8) imbedded in celloidin after fixation in Zenker's fluid followed by hardening in Müller's fluid.
9. Cell from the cerebral cortex of a brain (rat no. 9) imbedded in celloidin after fixation in Dahlgren's fluid followed by hardening in Müller's fluid.
10. Cell from the cerebral cortex of a brain (rat no. 26) imbedded in celloidin-paraffine after fixation in 30 per cent alcohol.
11. Cell from the cerebral cortex of a brain (rat no. 35) imbedded in celloidin-paraffine after fixation in the fluid of Lang.
12. Cell from the cerebral cortex of a brain (rat no. 29) imbedded in celloidin-paraffine after fixation in sublimate-acetic.
13. Cell from the cerebral cortex of a brain (rat no. 34) imbedded in celloidin-paraffine after fixation in Carnoy's fluid.
14. Cell from the cerebral cortex of a brain (rat no. 17) imbedded in paraffine after fixation in Ohlmacher's solution.
15. Cell from the cerebral cortex of a brain (rat no. 17) imbedded in celloidin-paraffine after fixation in Ohlmacher's solution.

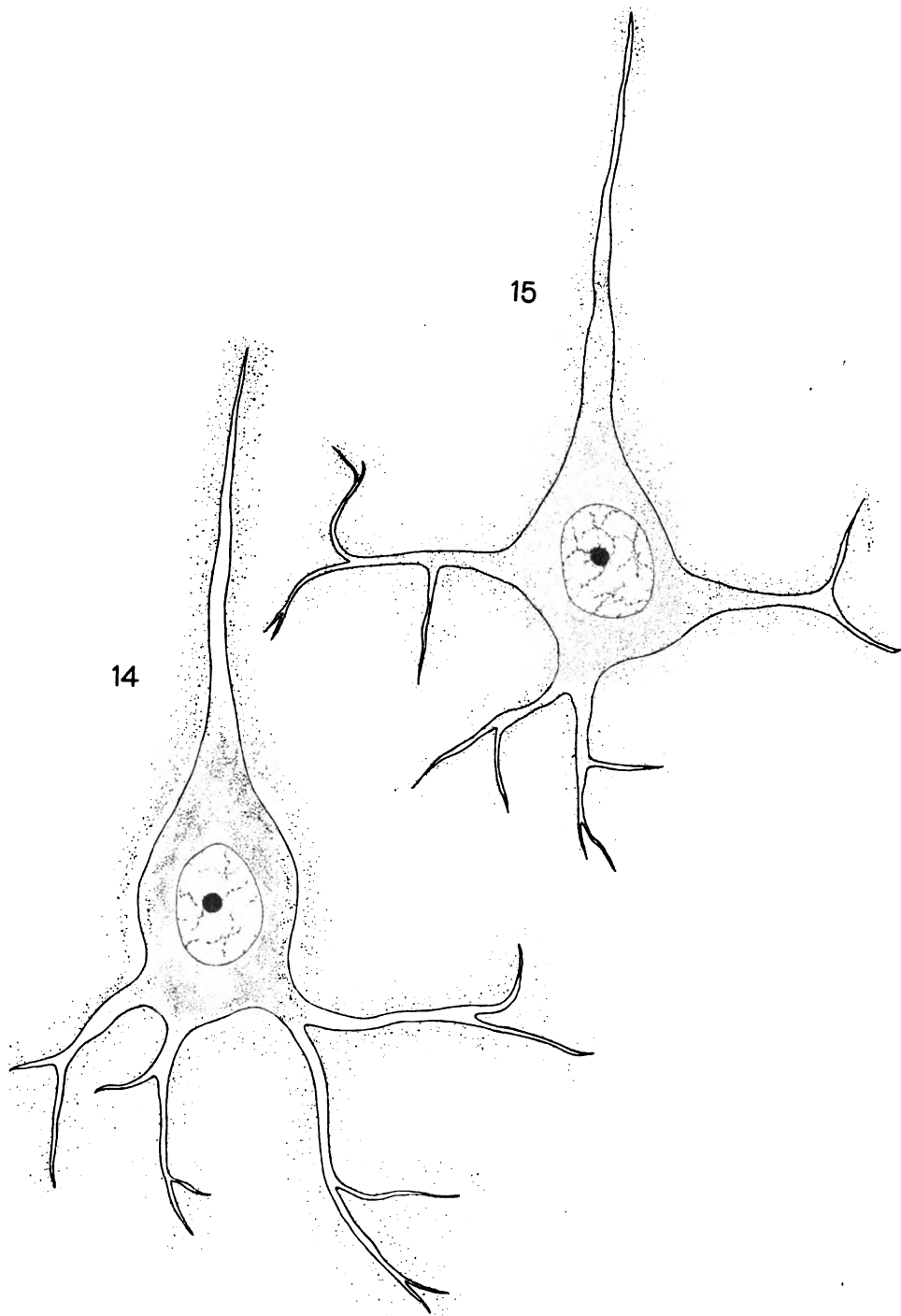












# THE CORTICO-SPINAL TRACT OF THE RAT<sup>1</sup>

JESSIE L. KING

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WITH TEN FIGURES

The pyramid tract fibers, carrying impulses from the motor cortex of the brain, have been the subject of much investigation both in man and animals but an interesting variation in their course in some rodents seemed worthy of study by the most approved method for tract work.

The earliest observations of its relations in rodents were made more than half a century ago by Ludwig Stieda. In his report of work on the brain of the mouse, he stated that the pyramids decussate into the posterior columns instead of the lateral as is usually the case and one of his figures through the decussation shows a decidedly X-shaped arrangement of the fibers.

Later Spitzka made a more extensive study on the "Comparative anatomy of the pyramidal tract" in which he mentions that in muridæ and in cavia (less pronounced) the pyramids decussate into the posterior columns. He does not indicate the method used but speaks of having had a series of transverse sections of a rat's brain prepared with especial reference to this inquiry.

The methods used by these investigators were inadequate for tracing tract fibers with accuracy and the use of the embryological method of Fleischsig was a decided step in advance. In 1889 von Lenhossék employed it on the mouse and guinea-pig, describ-

<sup>1</sup> After going to press I found a paper by Van der Vliet in *Anatomischer Anzeiger*, Band. xxix, p. 113, Ueber den Verlauf der Pyramidenbahn bei niederen Säugetieren in which the pyramidal system of the rat is investigated. His results and mine are in close agreement.

ing and illustrating the pyramid tract in the cervical, dorsal and lumbar regions of the cord. A little later Von Bechterew used the same method on the rat and guinea-pig. His paper gives a very brief description and his results are similar to those of Von Lenhossék on the two rodents he examined. He mentions the well developed pyramid tract of the rat and says that the nature of the decussation is apparent from a naked eye examination of an adult brain. Ziehen summarized the work done on rodents in his "Nervensystem" giving references to the literature. In 1909, Miss Reveley, working in the laboratory here and using the method of secondary degeneration with Marchi staining on the guinea-pig, confirmed in part the results of these early workers.

This latter method is recognized as the most accurate for such investigations. It has the advantage of giving positive instead of negative pictures and not only can the limits of a tract be definitely made out in section but, as in the decussation, individual fibers can be traced. I, therefore, selected it for my study of the pyramid tract in the albino rat. The work was done under Professor Simpson's direction, to whom I am indebted for many suggestions.

#### METHOD OF INVESTIGATION

This consisted in removing the cerebral motor cortex on one side and tracing the resulting Wallerian degeneration by the Marchi method.

The operations were performed under complete ether anæsthesia and with strict antiseptic or aseptic precautions. The cerebral cortex was exposed on one side and an attempt was made to localize the motor cortex by electrical stimulation using both the uni-polar and bipolar methods. On account of the small size of the brain, however, the results obtained were not constant and therefore unsatisfactory, and lesions were made by scraping away and cauterising the gray matter on the lateral aspect of the anterior half of the hemisphere, extending over the superior border onto the mesial aspect to some extent, so as to include the area from which the pyramid fibres take origin. The lesion in every case

was probably more extensive than the motor cortex but since the cortico-spinal fibres mainly were under investigation, this fact is not of material importance.

Eight animals were experimented upon. They were allowed to live from ten to fourteen days after the operation and then killed with chloroform. The brain and spinal cord were removed, placed in a 3 per cent solution of potassium bichromate, and at the end of three weeks segments from different levels were stained by van Gehuchten's modification of the Marchi method and then imbedded and cut in paraffin. Before the brain was sliced into segments, photographs were taken showing the extent of the lesion.

#### DESCRIPTION OF SECTIONS

Nearly all of the sections drawn are from the brain and cord of one rat having a small lesion but in every case the degenerated area is the same as in subjects having more extensive brain lesions.

The fibers of the pyramid tract descend in the internal capsule and form, to a large extent, the crusta of the mid-brain. In the section taken at this level (fig. 1) the degenerated area occupies a little more than the mesial half of the crusta but there are a few scattered fibers in the lateral portion. No fibers can be seen to pass backward from the crusta as are found in the cat and dog and the degeneration is confined to the side of the lesion.

In the middle region of the pons (fig. 2), the whole mass of the pyramid bundles is uniformly degenerated. On the anterior aspect of these bundles some fine degeneration is visible among the cells of the nuclei pontis. Still farther posteriorly (fig. 3) the transverse fibers of the pons break up the tract into bundles flattened antero-posteriorly which on the lesion side are extensively degenerated.

Still lower, just at the beginning of the decussation, (fig. 4) the anterior pyramid in transverse section presents an area throughout which the degenerated fibers are uniformly scattered. The decussation begins at the level of a group of cells representing

probably the inferior olivary nucleus. The fibers pass backward in separate small bundles at an acute angle to the raphe. They intermingle in crossing with those of the other (sound) side and passing through the grey matter, terminate in the base of the funiculus cuneatus. In passing downward (fig. 5), the decussating bundles become larger and larger following the course above described. One bundle of fibers becomes lost in the grey matter of the dorsal horn and a single isolated bundle was observed to

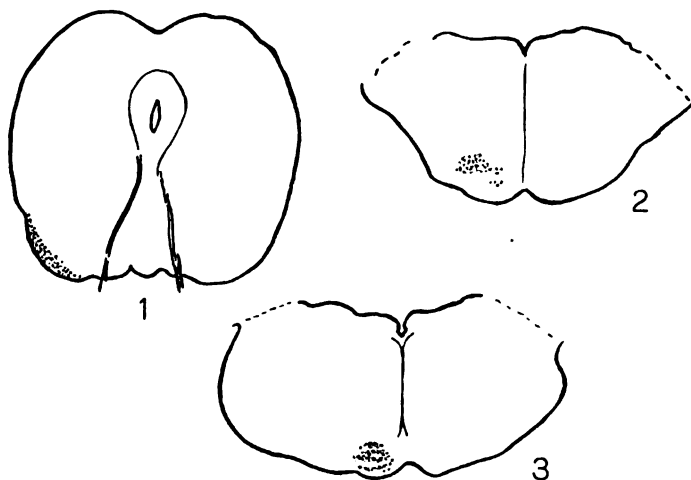


Fig. 1. T. S. Mid-brain at the level of the third nerve.  $\times 5$  diam. The dotted area in this and the following figures shows the extent of the degeneration.

Fig. 2. T. S. Mid-pons.  $\times 5$  diam.

Fig. 3. T. S. Lower pons.  $\times 5$  diam.

pass backward more laterally than the others and after turning downward, to terminate in the grey matter. At the lowermost portion of the medulla (fig. 6), the fibers of the pyramid tract occupy the anterior angle of the funiculus cuneatus, the lower part being separated from the fibers of the opposite side by the posterior median septum. No degenerated fibers are observed to pass to the posterior column of the same side, all cross the raphe.

In the cervical region of the cord (fig. 7), the area of degeneration is strictly confined to the column of Burdach; all the fibers have crossed and there is no degeneration on the side of the lesion and no degenerated fibers can be traced into the grey matter. The dorsal, lumbar, and sacral regions (figs. 8, 9, and 10) show no differences of significance from the cervical region except in the gradual decrease of the number of fibers making up the tract. In the lower sacral region they completely disappear.

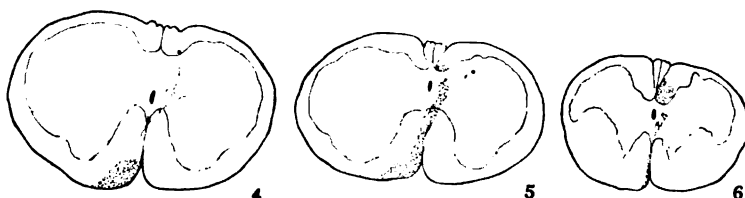


Fig. 4. T. S. Medulla oblongata at the beginning of the decussation.  $\times 6$  diam.

Fig. 5. T. S. Medulla oblongata at the middle of the decussation.  $\times 6$  diam.

Fig. 6. T. S. Medulla oblongata near the end of the decussation.  $\times 6$  diam.

In order to obtain a better idea of the relative decrease in the number of pyramid tract fibers, I have counted those degenerated, both coarse and fine, in the medulla above the level of the decussation and in some segments of the cord with the following results:

	FIBERS
Medulla oblongata, directly above the decussation.....	901
First cervical segment directly below the decussation.....	354
Third cervical.....	254
Seventh cervical.....	212
Sixth dorsal.....	169
First lumbar.....	155
Fifth lumbar.....	82
Upper sacral.....	71



According to these numbers, the largest proportion of fibers does not descend below the medulla and must therefore end in its grey matter.

So far I have been unsuccessful, except in the region of the decussation referred to (fig. 4), in tracing any fibers into the grey matter, although in one subject, I serialized the brain below the sub-thalamic region and the entire cord, while in all of the others some segments of the cord and the larger part of the medulla were serialized.

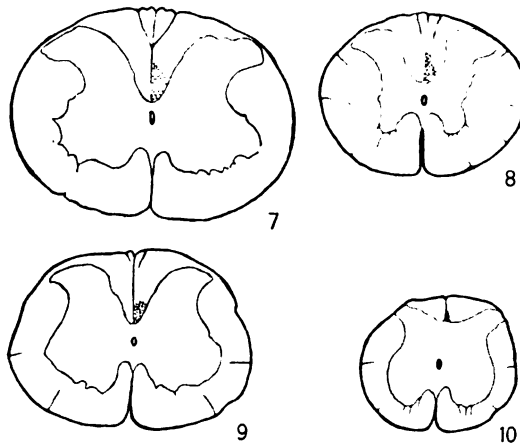


Fig. 7. T. S. Lower cervical region.  $\times 10$  diam.

Fig. 8. T. S. Mid-dorsal region.  $\times 10$  diam.

Fig. 9. T. S. Lower lumbar region.  $\times 10$  diam.

Fig. 10. T. S. Sacral region.  $\times 10$  diam.

#### CONCLUSION

In relation to the difference of opinion which exists with regard to the termination of the pyramid tract fibers in the grey matter of the spinal cord, their position in the posterior column is significant and would appear to give some support to the view held by Schäfer and Von Monakow that these fibers end immediately in relation to nerve cells situated in the posterior horn

and that the functional connection with the large multipolar cells of the anterior horn is established through an intermediate neuron. To be sure no degenerated fibers could be traced into the grey matter but this is not difficult to understand since the Marchi method fails as soon as the nerve fiber loses its myelin sheath.

Another point of interest is the fact that the decussation is complete. In all animals in which the crossed pyramid tract runs in the lateral column—rabbit, cat, dog, monkey, ape, man—there exists also a homolateral tract either in the lateral or the anterior column. This appears to be entirely absent in the rat. When compared with the cortico-spinal tracts in the rabbit, cat, and dog, which I have had the opportunity of examining in Marchi preparations in the laboratory, there is a striking paucity of fibers in the rat so that in this animal the so-called primary motor path is probably only of secondary importance.

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# A DEMONSTRATION MODEL OF THE BRAIN-STEM

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WITH TWO FIGURES

For a number of years a class model of the spinal cord<sup>1</sup> has been used in this laboratory in teaching the microscopic anatomy of the central nervous system. The model has proven so efficient in leading the student to an appreciation of third dimensions, and in aiding him in the formation of an accurate conception of the anatomical and physiological relations of the various nuclei and axone pathways, that it has seemed profitable to the writer to undertake the construction of permanent models of the more complex parts of the central nervous system.

The model herein described, (Fig. 1,) is of the brain-stem, showing the nuclei and central connections of the cranial nerves excepting the first and second. The plan of construction was suggested by the model already in use, which consists of wires representing the principal axone pathways passing through enlarged sections at different levels of the spinal cord. The material used and the mechanical devices employed, which in many cases were suggested by Mr. E. F. Miller, one of the assistants in this laboratory, have so demonstrated the feasibility of model construction as an aid to the study of complex parts of the encephalon, as to warrant the preparation of this paper.

The first step in construction was the selection of sections suitable for enlargement. These were chosen from normal trans-

<sup>1</sup> Hardesty, I. *The Johns Hopkins Hospital Bulletin*, vol. 17, no. 179, Feb., 1906.

verse sections through the following regions of the brain-stem: The superior corpora quadrigemina, the inferior corpora quadrigemina, the middle of the pons, the inferior border of the pons, the middle of the inferior olives. Each section was enlarged to the same extent by the Edinger projecting apparatus and a tracing of its outline taken on a large piece of paper. Not only was the contour carefully reproduced, but also the outlines of the gray substance and the principal nuclei and pathways. The paper sections were then cut along the edges of the figure and were ready for reproducing in a permanent form.

In seeking a material for the reconstruction of the sections, the relative qualities of wax and wood were considered: and finally both were discarded in favor of "fibre," used by electrical workers. Wax is not durable in climates subject to extreme temperatures, but after a time the surface cracks and gives a rough appearance to the model. It is easily worked and receives the impression of tools readily; but owing to this very quality it must be handled with greater care than a serviceable model should necessitate. Wood is better than wax, especially the three-ply boards used by cabinet makers, which will not warp; but it is subject to the disadvantage of flexibility; and it must be worked with care to prevent splitting. Fibre was chosen because it is not subject to warping; it is hard, tough, and homogeneous and will not split or chip on the edges; and it will stand the heat. It is very easily cut with a scroll-saw and the edges may be trimmed and smoothed with a file. In making holes the drill leaves a perfectly smooth bore.

Each paper section was laid on a large sheet of fibre three-eighths inch in thickness, and tracings were carefully made. The tracings were then cut with a scroll-saw into sections and the edges of each section were trimmed and smoothed with a file. The fourth ventricle and aqueduct of Sylvius were also cut out with the saw, leaving the sections with the areas for the nuclei and pathways marked out on the surfaces. These areas were then painted to distinguish them from one another, the same general scheme of colors being used as in the model of the spinal cord,

thus correlating the two models. During this procedure Spalteholz's "Atlas of human anatomy," and "Morris's anatomy," Part 3, were largely used. Nuclei and gray matter in general were painted white; sensory neurones through the lemnisci, and afferent fibres through the cranial nerves, red; motor fibres through the pyramids, and efferent fibres, blue; cerebello-spinal, cerebello-cerebral, and transverse pontine pathways, different shades of green; association pathways and reticular formation, brown. The backs and edges of the sections were painted black. Quick-drying enamel paint was found to be most practicable.

When the paint had thoroughly dried, holes were bored through the sections in the different areas to permit the passage of wires, care being taken to preserve a proper space between the holes in order that the model when strung with wires should not be too compact. In all the pathways it was planned to string only such wires as would show the principal directions and connections of the neurones. In boring, a drill was selected that would make a hole slightly larger than the wire, so as to leave room for wedging the wire into the hole with a small wooden peg. Enough holes were drilled in each section before setting up the model; and in cases where too many had been drilled, the supernumerary holes were plugged with a small wooden peg.

Sections of the inferior olives were cast and fastened to their respective sections before the model was finally set up. The method of casting was as follows: A paper pattern of the olive was made from the original paper tracing, and this was laid on a plate of paraffine three-eighths of an inch in thickness. Then with a sharp edged tool a paraffine pattern was cut from the plate. This was embedded in plaster of paris, and after the mold had set the paraffine was melted out. The casting was made with Wood's metal and pegs of the same material were fastened to it at appropriate places for attaching it to the section. Four such castings were made, two of the section through the middle of the olives, and two through the superior tips, as shown in Fig. 2.

Next a base of two-inch sugar pine was chosen and prepared for mounting the sections. Two one-quarter inch brass rods were

set into the dorsal edge of each section; and the sections were placed vertically on the base at equal distances apart and supported by the brass rods.

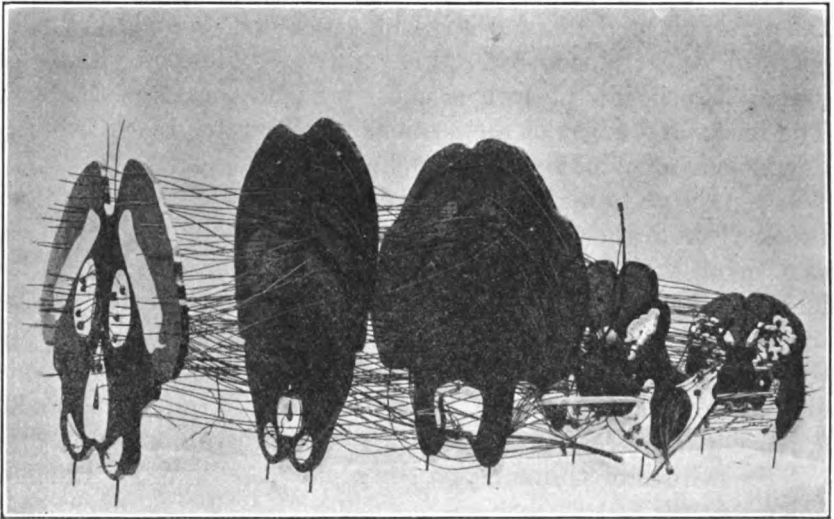


FIG. 1. Photograph of model of brain-stem in profile.

With the mounting of the sections the model was ready to receive the nuclei of the spinal tract and solitary tract, which, in order to represent best their extension through two sections of the model, were cast in metallic rods. Aluminum was used to insure lightness in the model. Molds were made in molder's sand from wooden patterns whittled to the required shape; and into these the molten metal was poured. After a few trials very good castings were obtained; and the writer contemplates a more extensive use of aluminum in future models, even in the wires and cell-bodies. In any laboratory provided with a large iron spoon and a blast-lamp a sufficiently high temperature can be produced to melt the metal. After smoothing them with a file the castings were tightly inserted in their proper positions through holes in the sections. The nuclei ambiguus of the vagus and glosso-

pharyngeal were constructed of rods fastened perpendicular to the section. The superior olives were fashioned from pieces of wire gauze to insure lightness. From each piece of gauze was cut a circular sheet, the edge of which was deeply notched in several places by cutting out triangular pieces. When the edges of these triangles were brought together and soldered, an oval structure was formed which satisfactorily represented the superior olivary nuclei, fig. 2, S O. The structures were suspended in position by the wires representing the corpus trapezoideum, passing between the superior olives and the lateral lemniscus.

The next step in construction was representing the principal azone pathways. No. 18 soft copper wire was used because of its flexibility and the consequent ease with which it could be bent in stringing it through the sections. The wire was first stretched to remove all kinks, and then inserted into the holes through the sections. Care was taken to avoid the use of too many wires, in some cases one or two being sufficient to represent the pathways. Each wire was fastened in the model by driving a small wooden peg into the holes in the end sections through which the wire passed. Not only were the wires by this means made permanent, but the sections were properly supported, and the entire model rendered compact and rigid. The stringing of the wires of the cerebro-spinal pathways was completed first because they give off but few collaterals, which were soldered to the wires later. The cerebello-cerebral and cerebello-spinal pathways were also strung in and the cerebellar ends soldered together within small metallic rings to form the superior and inferior cerebellar peduncles, fig. 2, C P. The middle cerebellar peduncles were constructed later, after the cell-bodies and telodendria of the transverse pontine neurones had been attached to the fibres. In stringing the fibres of longer course, care was taken to preserve as nearly as possible the contour of the brain-stem. Fibres of the lateral lemnisci, associational, commissural, and arcuate fibres, were inserted in the model later, because to them cell-bodies and collaterals or end-brushes were to be fastened.

Cell-bodies of the sensory neurones were fastened in the following way: A strip of lead, flat on one side and rounded on the



other, was molded by running molten lead into a wooden trough of the required shape. The strip was laid on its flat side, and pieces about 8 mm. long were cut from it with a saw. Each piece was then deeply notched on its flat side lengthwise to receive the end of the wire axone. The axone was fastened to the cell-body by first flattening the end of the wire with a pair of pliers, then inserting the end in the notch on the cell-body, and finally closing the edges of the notch over the wire by tightly squeezing the sides of the leaden cell-body with the pliers. The cell-body was then smoothed with a file to reduce it to a hemispherical or slightly oval shape. Cells of this character were used in the sensory nuclei and for associational and commissural neurones. The cell-bodies of the motor neurones were cast in Wood's metal. A wooden mold was prepared of a stellate shape, and into this the molten metal was run. The rough cast was then smoothed with a hot iron; and a groove to receive the axone was made in one side by allowing the hot iron to rest for an instant on one surface. The axone was fastened to the cell-body as in the case of the sensory cell, except that the metal was closed about the wire by melting it with a hot iron.

Telodendria and collaterals were constructed of eight-strand picture wire as in the previous model,<sup>2</sup> by tightly twisting the wire and fraying out one end to represent the end-brush. But they were soldered to the axones by cleaning the contacts with sand-paper or a file, moistening the contacts with zinc chloride, and touching them with the tip of a hot soldering iron bearing a small globule of solder. In every case it was aimed to produce an even juncture between the picture wire and the copper wire. Branching collaterals were made by untwisting a length of picture wire, separating the strands into two bundles, and retwisting these, leaving the ends frayed to represent end-brushes. Collaterals and telodendria on neurones of longer course were soldered on after the wires had been fastened into position in the model. On almost all neurones of shorter course the wires could be pro-

<sup>2</sup> Hardesty, I. *The Johns Hopkins Hospital Bulletin*, vol. 17, no. 179. Feb., 1906, p. 47.

vided with their collaterals and end-brushes before being inserted into the model.

Transverse and arcuate fibres, and telodendria about nuclei on the sections, were fastened to the sections with small wire staples. The neurone was bent into the required shape and laid

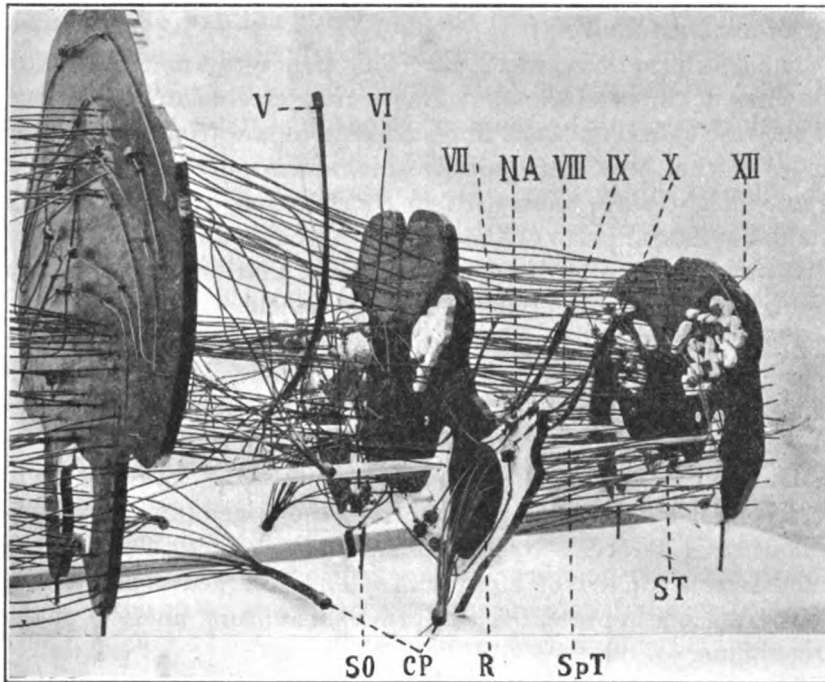


FIG. 2. Photograph of region of model at level of inferior olives. SO = Superior Olive, CP = Cerebellar Peduncles, R = Restiform Body, ST = Solitary Tract, Sp T = Spinal Tract, NA = Nucleus Ambiguus. V, VI, VII, VIII, IX, X, XII Cranial Nerves.

on the section. Then at two points widely separated on the axone holes were bored through the section, into which were inserted staples of bent copper wire, firmly claspings the neurones to the section. The staple was fastened into place by a small wooden peg driven into the hole; and the ends of the staple were cut off

even with the back of the section with a pair of pliers. Cerebello-olivary fibres were fastened to the inferior olives by melting a small piece of Wood's metal over the wire laid close upon the olive. Fibres of the corpus trapezoideum were fastened to the superior olives by passing a strand of picture wire through the gauze and over the axone-wire and soldering the ends.

Some of the features of the model that require more descriptive detail are the following:

The restiform body, fig. 2, R. This structure was represented by wires of the cerebello-spinal fasciculus, cerebellar connections of the fasciculus cuneatus, fibres passing to and from the inferior olives of the same and opposite side, and a portion of Gower's tract. Eight wires were used in construction, passing through the dorso-lateral parts of the sections through the middle of the olives and the inferior border of the pons, and bending dorsally and laterally as the inferior cerebellar peduncle.

The Solitary Tract and its recipient nucleus, fig. 2, ST, were represented by several fibres from the vagus and glossopharyngeus sending off collaterals at intervals, and terminating in end-brushes about the nucleus represented by an aluminum rod.

The Spinal Tract and its recipient nucleus, fig. 2, Sp T, were represented by descending fibres from the trigeminus nerve terminating at intervals by collaterals and telodendria about the nucleus constructed of aluminum. The enlarged cephalic end of the nucleus represented the principal sensory nucleus of the trigeminus.

The Nucleus Ambiguus of the glosso-pharyngeal and vagus, fig. 2, NA, was represented by a rod bearing motor cell-bodies of efferent fibres of these two nerves. These fibres were shaped to pass dorsally and mesially from the nucleus, bending laterally to meet the afferent fibres of the nerves on their way to the solitary tract and nucleus ala cinerea. The fibres were soldered together into two bundles and capped with a short length of copper tubing.

The motor nuclei of the cranial nerves were represented in all cases except those of the vagus and glosso-pharyngeal by single large stellate cells with their efferent fibres attached. In con-

structing the trochlear nerves which decussate in the anterior medullary velum and emerge on the dorsal surface of the mesencephalon the two efferent wires were bent about the central grey substance and soldered together at the point of decussation which was fastened to the section by a wire staple. The nucleus of the mesencephalic root of the trigeminus was represented by a wire passing through the two anterior sections of the model to join the fibre from the principal motor nucleus of the trigeminus, just ventral to its principal sensory nucleus. The motor nucleus of the facial nerve was represented by a single cell placed on a line with the motor nuclei of the trigeminus and glosso-pharyngeal. Its efferent fibre was bent to course about the nucleus of the abducens as the genu facialis, finally to emerge from the inferior border of the pons in company with the pars intermedia of the facial.

The Red nucleus, nuclei of the superior and inferior colliculi, nucleus ala cinerea, nucleus vestibularis, nucleus of the lateral lemniscus, and nucleus fasciculus cuneatus were represented by areas painted white on the sections, containing cells and telodendria of connecting fibres.

No attempt was made to show all the associations between the different nuclei, for fear of rendering the model too complex and unwieldy; but the principal connections were shown, and those most useful in working out the various reflex pathways. After all the nuclei and pathways were completed, the connections were made by soldering collateral fibres with a soldering iron.

After the construction of the model was completed, the wires of the different pathways were painted colors corresponding with the areas of the sections through which they passed. Wires used in supporting nuclei or fibres were distinguished from axonewires by a coat of black enamel paint. A second coat of paint was given to all the fibres and to the backs and edges of the sections. Wires were straightened and the fronts of the sections were touched up where the paint was scratched off during construction.

The dimensions of the model are as follows: length of base 36 inches, height 17 inches, width at the section through the middle of the pons 17 inches. The model is light, easily carried from one room to another, perfectly firm and tight, and likely to stand the usage of students observing reasonable care.

In conclusion, acknowledgements are due to Professor Moody, at whose suggestion the construction of the model was undertaken.

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## NOTES ON STAINING METHODS

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The following adaptation and modification of well known methods of microscopical technic have been found useful in the preparation of specimens for classes in histology.

*Method for staining cartilage:* In staining cartilage for histological examination, especially cartilage in which bone is forming the brilliant color contrast given by alum hæmatoxylin and eosin, the blue cartilage matrix, the red connective tissue of the perichondrium, red protoplasm, and red bone-matrix—makes this combination a valuable stain. One fault lies in the fact that frequently the cartilage matrix takes the alum hæmatoxylin stain before the nuclei of the cells, especially in tissues which have been kept some time in alcohol; to stain the nuclei necessitates the over-staining and consequent clouding of the cartilage matrix. By staining the nuclei first by Heidenhain's iron hæmatoxylin method, then the cartilage matrix lightly with alum hæmatoxylin, and finally adding the eosin, all the various elements are brought out clearly.

*Staining of connective tissue fibrils:* Mallory, in describing his method for the differential staining of connective tissue fibrils, states that the tissue must be fixed in Zenker's fluid. Not infrequently it is desirable to stain by this method tissues already fixed in 10 per cent formalin or in other fixing fluids, and fairly satisfactory results may be obtained by merely placing the block of tissue, or the sections, in Zenker's fluid before staining. Sections may remain in Zenker's stock solution (the addition of acetic acid

is not necessary) for an hour or more; blocks of tissue are treated by Professor Mallory as fresh material, and are run through the usual routine of Zenker fixation. The mordant action of the constituents of Zenker's stock solution seems to be necessary for the proper differentiation of the stain. This suggestion is offered only for those cases where it is desired to use this stain on previously fixed tissue, as the differentiation is not quite so sharp and clear as when tissue originally fixed in Zenker's fluid is used.

*The staining of segmentation stages of ova:* Mallory's connective tissue stain may also be used in the study of segmentation stages, as tried recently in this laboratory with some early stages of snakes (*Eutaenia radix*) after Zenker fixation. While the color reactions are not yet understood, they are so remarkable that it seems probable that they will be of value. The chromatic material of the nuclei and the chromosomes are dark red; the protoplasm is purplish blue (instead of red, as in the adult). Centrosomes and the spindle threads show a dark purple; the vitelline membrane is blue. The yolk is the most remarkable; yolk granules are of a yellowish red, but the white yolk and the partially digested granules are pale blue. All the color contrasts are beautifully clear.

*Staining of heart muscle:* A simple method of staining cardiac muscle to show the nuclei, striations, and intercalated disks, or "Schaltstücken" of Heidenhain, is by the use of Mallory's phosphotungstic acid hæmatoxylin. The nuclei and disks stain dark purple, the striations a little lighter, and the protoplasm pale purple. A counter-stain of Orange G may be added to bring out the connective tissue elements, without injuring the stain, if the precaution is taken not to leave the section longer than necessary in alcohol. Heidenhain states that corrosive sublimate fixation is probably a *sine qua non* for successful staining of the heart elements; but I have found that tissue fixed in 10 per cent formalin reacts to this stain, though perhaps the results are not quite so clear as with Zenker fixation.

*The Cox method:* With regard to the manipulation of Cox-Golgi preparations for the study of nervous tissue, the following may be said: Cox states in a note to his paper: "Nur bei kleinen Stückchen und schnellem Verfahren kann Celloidineinbettung ohne grossen Nachtheil für die Imprägnirung zur Anwendung kommen," since alcohol endangers the impregnation; and most other writers state that alcohol must be avoided as much as possible and advise imbedding in celloidin by dehydrating for a few minutes in absolute alcohol and then placing in a thick solution of celloidin for a few minutes. My experience has not agreed with these statements.

Finding that pieces of tissue imbedded thus rapidly were rather supported by a coating of celloidin than permeated by the solution, and that, although they could be cut, the sections crumbled and were very difficult to handle, I tried longer and longer periods in alcohol and celloidin until finally the material was submitted to the ordinary steps of celloidin imbedding, remaining in thin celloidin for a week or more, and an equal time in thick celloidin. These thoroughly permeated sections showed no loss of impregnation that could be detected by comparison with sections of the same tissue imbedded rapidly. This year I have used material which was slowly imbedded and blocked in 1904, and kept since then in 80 per cent alcohol; the impregnation is as full and as dark as ever, there is no deposit of mercury one month after mounting under a cover glass without heating. These thoroughly imbedded sections may be easily stained with hæmatoxylin and eosin, without injuring the impregnation: such stained specimens are especially useful in the cortex of the cerebellum, to show the distribution of the axons of the "basket-cells," etc. In my experience the stained specimens show less of the mercury deposit after several months than do specimens without stain.



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# INTRA VITAM STAINING WITH METHYLENE BLUE

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The purpose of this paper is not to give a detailed account of the various modifications of this important technic, it is rather to state clearly the particular methods which I have found most suitable and to point out the pitfalls a beginner has to avoid. That this technic is so little used and that it has been so unsuccessful in the hands of many seems sufficient justification for the account.

In 1885 Ehrlich drew attention to the fact that if a solution of methylene blue in physiological salt solution be injected into the blood vessels of a living animal the nerve cells and their processes showed an affinity for this dye. The tissue when removed and examined under the microscope usually shows no nerves, but on exposure to the air these gradually appear. The reaction depends on the fact that the coloring property of a dye is due to the presence of a group of atoms with a marked affinity for hydrogen. The introduction of hydrogen into this unsaturated radical results in a loss of color;<sup>1</sup> but this leuco-combination can be easily oxidized by the air to the original color. One may suppose that the "vital color," methylene blue, circulating in the blood is selected by certain cells by an extractive process, the "ausschüttelungs process,"<sup>2</sup> involving a reduction to the leucobase by the addition of hydrogen as a result of functional activity or post-mortem change. Exposure to the air oxidizes this to the original dye. It is, however,

<sup>1</sup> Nietzki, R.—*Chemie der organischen Farbstoffe*, Berlin, 1901, pp. 2 and 3.

<sup>2</sup> Ehrlich, Paul—*Collected Studies on Immunity*, New York, 1906. Article 34. The relation existing between chemical constitution, distribution and pharmacological action.

not sufficiently anchored in the cell, but can be fixed there by the action of certain acids which precipitate it in an insoluble form.

The importance of this "vital staining" reaction was quickly recognized and it is now a procedure which has found extensive application in the histology of the nervous system. Failure to obtain this selective action is not uncommon and arises from many causes, for instance, the difficulty of obtaining fresh tissue, the particular part examined, the fixation and subsequent treatment requisite to insure dehydration. The adequate saturation of the tissue with the dye, its reduction in the nerve and its subsequent oxidation are easily accomplished. In regard to the freshness of the tissue, the sooner after death it is obtained, the better the result. Animal tissue can usually be obtained immediately on death or very soon after. In man it is remarkable how many hours *post mortem* the nerves and their endings react to the dye; in some cases I have obtained satisfactory results six to eight hours after death, when the body has been kept in a cold chamber.

In some tissues the nerves react to the dye more readily than in others. Thus they are easily obtained in muscle, and here the motor endings appear more readily than the sensory endings or the vaso-motor nerves. Endings are easily demonstrated in the tongue and epiglottis but with more difficulty in the lung and in the liver. While this can be explained to some extent by the condition of the blood supply and the variety of nerve, it appears to me that some substance is often present around the terminal ending interfering with the reaction. The accessibility of oxygen to the reduced dye has always to be considered; thus the nerves of the skin stain with difficulty when the epithelial surface is exposed to the air; but if cut transversely and so exposed, the blue quickly appears.

The part of the technic in which one meets with the greatest difficulty is the process of dehydrating. The fixed dye is extremely soluble in all grades of alcohol excepting absolute. As the removal of a minute amount of the dye from the fine nerve ending is detrimental to a satisfactory result, it is to this part of the procedure that most care has to be directed.

The kind of methylene blue used is of importance. There are

many varieties of this dye; as it comes into commerce it is a zinc chloride double salt. For vital staining this dye is useless. A zinc-free salt is requisite; of these the one that answers best is the methylene blue "nach Ehrlich" prepared by Dr. Grüber of Leipsic. Another which gives fairly good results is the medicinally pure methylene blue.

Absolute cleanliness of the vessels used cannot be too strongly insisted on. The slides and instruments should be kept in physiological salt solution.

To get the dye to the nerve, there are three methods which I use, varying according to the animal or the part to be examined: I. The injection method—Ehrlich's method. II. The placing of a thin section on a slide and keeping it covered with the dye—Dogiel's method. III. The immersing of the tissue in a weak solution of the blue till it becomes permeated with the dye; then exposure to the air.

I. For the injection method it is generally recommended to use a  $\frac{1}{4}$ – $\frac{1}{8}$ % solution of methylene blue. This I consider too strong and too apt to color other tissues which will later be referred to, and so to obscure the nerves and their endings. I therefore use a solution never stronger than  $\frac{1}{20}$ %. A stock solution of methylene blue 0.5% in distilled water is made, and when ready to inject the following is prepared:

Methylene blue (0.5% sol.)—10 cc.

Salt solution (0.75% sol.)—90 cc.

Even this will often color other tissues too much and then the strength must be reduced. Before injecting, this solution is heated to slightly over 37° C. In small animals, *e.g.*, the white rat, it is most suitably injected into the aorta or heart; in large animals the vessel selected should be near the part to be investigated; thus for endings in the muscle of the eye, or of the tongue in a dog, the injection may be made into the common carotid.

Before injecting, the animal is given an anæsthetic and bled. A glass canula may be inserted into the artery and the fluid

injected from a flask; or a syringe with a blunt needle may be used. I prefer the latter; with a little practice one can so arrange that the syringe can be withdrawn from the needle fixed in the artery, refilled and applied to the needle without air getting into the vessels. I do not now wash out with salt solution. While recognizing that it is absolutely essential for satisfactory staining to have the capillaries and veins thoroughly empty of blood, I find that this is accomplished most satisfactorily and with less damage to the tissue by bleeding thoroughly and then during the injecting to open a neighboring vein and allow the methylene blue to flow through this exit till the solution escapes uncolored by blood and with its distinctive blue. Then I clamp the vein and fill well all the blood vessels. The test by which this can be gauged is that the tissue to be examined be distinctly blue; or if the tissue be too deep to be seen without dissection, for instance in the m. tensor tympani, that the surrounding parts are well colored. To get this result it may be necessary to clamp all the vessels connected with the part, thus in the head, all the vessels of the head and neck coming from the arch of the aorta as well as those entering the superior vena cava.

The injected part is now left untouched for 5 to 10 minutes; then the part to be examined is exposed. Free entrance of air to the tissues is essential; at times one exposes the part to be examined for a few minutes before removing pieces of suitable size for microscopic examination. This removal may be done with a sharp broad knife, scissors, or a Valentine knife. The section is placed on a clean glass slide moistened with salt solution and examined under the low power. It may be, that already nerves are to be seen; if not, the slide is laid in a Petri dish and placed in the thermostat at 37°C. From time to time the section is examined under the low power of the microscope, say every five minutes, till the nerves are sufficiently clearly seen, care being taken in the meantime that the tissue is kept moist by the application of the salt solution. The recognition of how soon the tissue may be regarded as satisfactorily colored is a matter of experience, but in each piece the nerves will be seen in various stages of coloration. As a rule the tissue should be fixed too soon rather than too late. If kept too

long the blue diffuses out and a blurred appearance results. If the nerves do not appear within one hour it may be regarded as useless to expect them. It is interesting here to note that the nerves do not invariably appear blue—they occasionally are of a violet or reddish purple. This opens an interesting field for investigation as to what has caused this transformation.

In frogs the injection can be best done from the heart or vena abdominalis. In cold-blooded animals the solution is not warmed, nor is the tissue placed in the thermostat.

As a modification of this, one may inject the solution directly into the part. This is very useful in man, injecting subcutaneously to examine the skin for nerve endings, injecting into the muscles for motor or sensory endings. It is an easy method of preparing class preparations, for example, to demonstrate motor and sensory endings as well as vaso-motor nerves in the m. sartorius of the frog. When so used I prefer the solutions  $\frac{1}{2}$  or  $\frac{1}{4}$  of the above strength. When the tissue is well colored the part is cut out, placed on a slide, examined and fixed in the usual way.

II. In the second method the piece of tissue freed from blood (if necessary by rinsing in saline solution) is cut to a suitable size, placed on a glass slide and its surface moistened with the  $\frac{1}{20}\%$  solution of methylene blue at  $37^{\circ}\text{C}$ . It is then laid in a Petri dish and placed in the thermostat at  $37^{\circ}\text{C}$ . in the case of warm-blooded animals and at room temperature for cold-blooded animals. It is examined from time to time and kept moist with the solution. To prevent it drying I usually raise the slide slightly above the bottom of the dish and keep a little distilled water or damp cotton wool in the dish. Within a varying time, up to 2 hours, the nerves appear.

III. The method which I have recently used very largely and which has proved more effectual than Method II for human tissue and for the organs of large animals such as the heart of the calf, is immersion of the tissue for a short period in a weak solution and then exposure to the air. The details applied to the heart of a calf are as follows:

a. With a Valentine knife a section is cut of the part to be examined about 1 mm. thick, the size otherwise being of no moment; some of my sections have been 3 to 4 cm. long and 1 to  $1\frac{1}{2}$  cm. in breadth.

b. This is immersed in a weak solution of methylene blue at 37° C. and placed in thermostat for 5 to 10 minutes or till the tissue is well colored blue. For this purpose I use

Methylene blue (0.5% sol.)	5.0
Salt solution... (0.75% sol.)	.95.0

Often a much weaker solution answers better.

c. The tissue is now taken out of the solution and placed on a glass slide in a Petri dish, moistened with the blue solution and placed in the thermostat at 37° C.

d. At a varying time the nerves appear, the interval varying with the time after death at which the tissue has been obtained. They may begin to appear as early as from 10 to 20 minutes or they may not appear for an hour.

The human heart may be treated in exactly the same way. The time at which the nerves appear varies with the time after death, the longer after death the slower the appearance; it also varies with the animal, thus in the sheep and ox they appear more slowly than in the calf. It will be noted that I use the weakest possible solution because since the nerves attract the dye more readily than do the other tissues, a clearer picture can thus be obtained.

#### FIXATION

The dye has to be fixed in the nerve, otherwise it quickly disappears. To secure this, one may employ either 1, the ammonium picrate method of Dogiel, or 2, the ammonium molybdate method of Bethe.

1. In the ammonium picrate method the tissue is placed in a saturated filtered solution of ammonium picrate (Grübler's) in water. Here it is left for from 4 to 24 hours according to its size. It is then transferred to a mixture of equal parts of glycerin and

the above solution in which it clears and in this it is mounted and examined. While there are certain cases in which this method is of great use, for example in the examination of teased preparations, yet for detailed study and accurate results it is inferior to the method of Bethe.

2. For routine work Bethe uses a 5% solution of ammonium molybdate. At first he recommended various additions to this solution, such as hydrochloric acid (a few drops), peroxide of hydrogen, osmic acid, etc. Experience has shown that these are superfluous and they are now but little used. I constantly fix with an 8% solution. The kind of molybdate used is of some importance. The large crystalline variety prepared by Merck or by Kahlbaum is to be preferred. The crystals are added to the distilled water in a flask; the mixture is heated, but not allowed to boil, till the crystals are dissolved. A large amount of the molybdate solution must be used. Before placing the tissue in this fixing agent it is advisable to cool the solution to about 35–40° F. by placing it in a cold chamber. Into this the tissue is placed and left over night in a cold chamber. It appears to me that the molybdate solution has an oxidizing effect, and so it is preferable to fix the nerves as they are coming out rather than after they have been out for some time. Unless this is done the picture is less sharp, due to the blue being fixed as it is diffusing out from the axis cylinder. The oxidizing effect of the molybdate will also account for the greenish yellow color so often seen, as for example in the muscle cell.

I have used a mixture of osmic acid and ammonium molybdate in order to obtain a combination of the blue axis cylinder with a brown coloration of the medullary sheath. The tissue is placed for 1–2 hours in the following solutions:

Ammonium molybdate (8% sol.).....	100 cc.
Osmic acid ( $\frac{1}{2}$ % sol.).....	10–15 drops

The amount of osmic acid must be just sufficient to stain the tissue a light brown. After the expiration of this time the tissue is transferred to an 8% molybdate solution in which it is left over



night. In using this technic I do not allow the nerves to appear well under the low power because the oxidizing effect of the osmic acid is sufficient to assist in transforming the leucobase.

#### WASHING.

The tissue must be washed to remove the molybdate. This may be done in one of two ways:

a. By using several changes of distilled water for 1 to 2 hours. It is a good plan to keep the water cool by having a piece of ice in it because in all laboratories the water is apt to be warm and the dye dissolves out, in small amount, it is true, but sufficient to spoil the final results.

b. Recently I have washed the tissue in ordinary cold tap water running very slowly, by which the time of washing is very considerably reduced to from  $\frac{1}{2}$  to 1 hour. Any means of shortening the time between fixation and embedding is of distinct advantage; the aim should be to get this done in one day.

#### ALCOHOL TO PARAFFIN.

The method of passing the tissues through alcohol is of the utmost importance. Alcohol, even 96 per cent, will extract the dye; in absolute alcohol the dye is practically insoluble. I have left well dehydrated tissue over night in absolute alcohol without a trace of the dye showing in the liquid. My mode of procedure is as follows: On removing the tissue from the water the surface water is removed with blotting paper. I do not press the blotting paper on it but simply lay the tissue for a few seconds on the paper. It is now immersed in 96 per cent alcohol and rinsed; then transferred to fresh 96 per cent alcohol for about 5 minutes, again transferred to 96 per cent alcohol for 20 minutes and again to 96 per cent alcohol for 1 hour. In all this procedure the alcohol is kept at a temperature of about 40 degrees F. in a cold chamber. The number of times this is done depends on the thickness of the tissue and the amount of shrink-

ing one wants to avoid; thus in some cases I have kept the tissue in 96 per cent alcohol altogether for from 2 to 4 hours.

Now it is transferred to absolute alcohol. It is best to have the first change of absolute in the cold chamber but after this it may be kept at the room temperature. It is well to remember that during the process of dehydration the absolute alcohol becomes diluted and that as the diffusion of alcohol in water is slow, one can then see a ring of color immediately surrounding the tissue. It is advisable to have thin pieces and to hurry the process on to xylol and paraffin, but absolute dehydration is most essential. Xylol is by far the most satisfactory clearing agent; most of my tissues about 1 to 2 mm. thick clear in about  $\frac{1}{2}$  hour.

The tissues may then be mounted in Canada balsam and examined; or they may be passed into paraffin. The paraffin used is a matter of choice. Lately I have immersed the tissue in soft paraffin in a vacuum bath for  $1\frac{1}{2}$  to 2 hours; then into hard paraffin for a minute, imbedding in hard paraffin. Once in paraffin the tissue is safe; I have cut paraffin blocks after 4 years and found the tissue in perfect condition.

We can briefly summarize these methods as follows:

#### I. Injection method.

1. Anæsthetise and bleed animal.
2. Inject into artery  $\frac{1}{4}$  per cent methylene blue till part well colored.
3. Cut out part and examine under low power of microscope; if no nerves then
4. Keep moist in thermostat at  $37^{\circ}\text{C}$ . till nerves appear—15 min. to 1 hour.
5. Fix in 8 per cent ammonium molybdate over night.
6. Wash in cold water for  $\frac{1}{2}$  to 2 hours.
7. Pass through several changes of 96 per cent alcohol at a low temperature for  $\frac{1}{2}$  to 2 hrs.
8. Pass through several changes of absolute alcohol for 1 to 2 hours.
9. Clear in xylol.
10. Mount in Canada balsam or embed in paraffin.

## II. Dogiel method.

1. Small pieces of tissue from animal recently killed, free from blood, are placed on slide and moistened with  $\frac{1}{2}$  per cent methylene blue, and kept at temperature of  $37^{\circ}\text{C}$ . till nerves appear—usually within one hour.
2. Fixation and after treatment as in method I.  
For modifications for cold blooded animals and for ammonium picrate fixation, see text.

## III. Immersion method especially suitable for human tissue and for the tissue of large animals.

1. Immerse as soon after death as possible a thin section of tissue freed from blood in a  $\frac{1}{4}$  per cent solution of methylene blue at  $37^{\circ}\text{C}$ . till permeated by blue, for 5 to 15 minutes.
2. Remove from solution, place on a glass slide and keep moist with the above solution at  $37^{\circ}\text{C}$ . till nerves appear,  $\frac{1}{2}$  to 2 hours varying with time after death.
3. Fixation and after treatment as in Method I.

## SOURCES OF ERROR

In all methylene blue investigations it must never be forgotten that while the dye is neurotropic it is not monotropic, otherwise one may be led to false deductions. In *intra vitam* staining in addition to nerve tissue there are colored by the dye elastic fibers, pigment and connective tissue cells, fat cells and muscle cells. Fat cells and muscle cells never cause any confusion; but the others require to be constantly kept in mind as a possible source of error. The elastic fibers especially if in single strands may at times look like nerves, but the absence of the typical varicosities and their mode of branching are sufficiently distinctive. This error is likely to occur only to a beginner or in unsatisfactorily stained tissues. The connective tissue cell

and the pigment cell with their branching processes have not infrequently been mistaken for ganglion cells. This mistake can only occur in smaller cells when the nucleus is either not seen or badly stained. The large ganglion cell with its distinctive nucleus and halo never presents any difficulty. The small pigment cells in which the nucleus has not appeared well, at times may resemble at first glance a ganglion cell; but the processes are different and can never be traced to the same length as in the ganglion cell nor do they branch with the same regularity. A comparison of a doubtful cell with a ganglion cell always will solve the doubt.

To some this account may appear too minute but I have seen so many failures from "slight alterations" or lack of attention to an apparently insignificant point that an excess of detail may well be pardoned. I have added no bibliography since this has been done so amply by Dogiel<sup>3</sup> in a recent article.

<sup>3</sup> Dogiel, A. S. Methylenblau zur Nervenfärbung. *Encyklopädie der Mikroskopischen Technik*. Bd. 2, Berlin, 1905.

## BOOK REVIEW

MEDICAL EDUCATION IN THE UNITED STATES AND CANADA. • By Abraham Flexner. With an Introduction by Henry S. Pritchett, Bulletin No. 4, of the Carnegie Foundation. 576 Fifth Ave., New York City. XVII. 346 pages.

This exhaustive report upon medical education, and upon the medical schools in the United States is comprehensive and timely, and will no doubt be of great value in bringing about desired reforms. It is divided into chapters touching all phases of medical education, and gives a detailed account of each medical school in the United States, all of which have been personally visited by Dr. Flexner. Throughout the report, anatomy is constantly alluded to, and every anatomist should be vitally interested in this report and should study it with care. The report as a whole shows that Dr. Flexner has full command of his subject. It is not destructive, but constructive, even admitting that he would destroy the weaker medical schools, which are a disgrace to American medicine. The reviewer is of the opinion that in general Dr. Flexner has been much too lenient in his criticism, for he praises whenever there is opportunity. The truth regarding the situation is by no means as rosy as he states it. After the ideal is reached, as he pictures it, the medical schools will still be far from being great productive centers—a dream which he holds out for New York City only. We may all continue to be optimistic, for five or six really great men will make a great medical school anywhere. It may be that the time is ripe for this possibility, and if so, really great medical schools will be found in a relatively large number of states.

Dr. Flexner classifies the medical schools according to their entrance requirements. The first class is formed of those medical schools requiring at least two years of collegiate work, twenty-five in number. In these schools the professor of anatomy usually has the degree of M.D., in some few instances the degree of Ph.D. He is nearly always a professional anatomist. Asexceptions, there may be mentioned Western Reserve of Cleveland and Cornell in New York City, in which schools the anatomists are active surgeons. "The practitioner usually lacks impartial and eager scientific spirit; he can at best give set hours to teaching, and these are not infrequently interrupted by the patient's superior claims; of course he has little or no time, and little zest for research." (Page 72). It may be added that even this little zest is not always possessed by the professional anatomist. Until it is, this argument, which is the strongest, will not always be accepted. Doctor Flexner is of the opinion that a uniform curriculum of hours in anatomy is neither feasible nor desirable. The endeavor to improve medical

education through an iron-clad prescription of hours is wholly mistaken. It cannot materially improve the poorer schools, and very seriously hampers the competent institutions. If teaching is in inferior hands, printed directions will not improve it. The prescribed curriculum is a useful staff in the hands of those who have not strength to walk alone. Fortunately in these better schools the practice varies widely, and Doctor Flexner gives all of the desired data relating to anatomy. He apparently approves of the elective system and in freedom for mature students. In the University medical school the studies may be concentrated, and need not be given out in small doses, as is the case in weaker schools dealing with immature students. In the former the beginning student devotes all his time to anatomy, and of necessity the staff must be on hand all day as in any other well equipped laboratory. The student should be permitted to work any time, according to Virchow's motto, and the teachers should be so devoted to their work that nothing will take them away from it. This is possible only with a research staff.

The schools of the second order are those having entrance requirements of graduation from an approved high school or less, about one hundred and twenty-five in number. These are classified as follows: 1, Those that by careful selection of students and extraordinary pains in teaching make the very most of the situation. 2, Those that are content to operate on a lower plane, but are still commercially effective. 3, Those that are frankly mercenary. The first group form a small minority. They are generally too good for the students they get—McGill, Toronto, New York, Syracuse, Jefferson, Northwestern, Tulane, Texas, St. Louis. The latter affords an excellent example of a brave, uphill contest, through the intensive cultivation of anatomy and physiology. Eycleshymer has here created a productive department which has invigorated the entire school. It may be of interest to presidents and clinicians to know that Eycleshymer is an anatomist, a Ph.D., and not a surgeon. I desire to emphasize this point because an extensive experience with both classes of officers makes this necessary.

In the second group anatomy is inert, and usually goes with surgery. There are usually no decent teaching facilities; material abounds, but the practitioner-anatomist does not make use of it. If he is a good man, practice calls him, and if he is a poor practitioner he usually does not know how to make best use of anatomical material. In such schools the anatomical laboratory is a mere dissecting room, in which the student is required to dissect portions of the cadaver under the guidance of an upper class-man, or recent graduate. Into none of these schools have modern ideas as to the conduct of the department of anatomy permeated. The story is too well known to continue. It would be a blessing to the nation if all of these schools were abolished.

There is, however, a third group which is still worse. Their main weapon is the quiz-compend. Doctor Flexner states that it is stretching the term laboratory to use it in connection with such schools. They are closely associated with homeopathy, osteopathy and ignorance.

They are institutions of quackery. No equipment will ever improve them, and they especially keep our profession in bad repute. No decent doctor can afford to have anything to do with them.

It is impossible in the pages of the *ANATOMICAL RECORD* to enter more fully into this valuable and conservative report. It is, however, sincerely hoped that all anatomists who may not have seen it may procure a copy for thorough study. That its publication caused so great a commotion is a most healthy sign.

It falls upon all anatomists to prepare for the near future. There will be need of many trained anatomists, who are at the same time scientific investigators. Never was the demand for such greater than now, and it is reasonable to hope that the career of the teacher of anatomy will soon be greatly bettered. The research departments of anatomy in this country should give every encouragement to students inclined toward anatomical research, in order that we and our successors may do our part in the medical reform which confronts us. How great the field is, the report of Doctor Flexner has pointed out.

FRANKLIN P. MALL.

## DEFOREST'S FORMULA FOR "AN UNSYMMETRICAL PROBABILITY CURVE"

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In presenting a long-forgotten investigation by E. L. DeForest ('82-'83) on "an unsymmetrical probability curve," the writer wishes to call attention to the fact that the first systematic analysis of the subject was attempted by DeForest and as a result he obtained a formula which is identical with that for Professor Pearson's ('95) generalized probability curve. DeForest suggests further that by retaining the higher derivatives a more general formula, of which the formula already found will be a particular case, may be obtained from his original differential equation. Thus DeForest's investigation is not only interesting from an historical standpoint, but still more from the fact that the same formula, though in different terms, has been derived from entirely different methods of analysis by Professor Pearson. This fact furnishes good evidence as to the validity of Professor Pearson's theoretical assumption.

As the investigation was published a number of years ago, the original paper by DeForest is difficult to obtain, and so, for the reader who is anxious to see the method of mathematical analysis adopted by him, I venture to present in the following pages some of the important points which directly concern the derivation of his final formula. I shall also add a mathematical process of transformation of Professor Pearson's formula to that of DeForest. For numerous other important and interesting points, the reader must refer to the original memoirs.

DeForest employed this reasoning:  
Let the following be a given polynomial

$$\lambda_{-m} Z^{-m} + \dots + \lambda_{-1} Z^{-1} + \lambda_0 + \lambda_1 Z^1 + \dots + \lambda_m Z^m. \quad (1)$$



Its expansion to the  $\kappa$  power may be written

$$l_{-\kappa m} Z^{-\kappa m} + \dots + l_{-1} Z^{-1} + l_0 + l_1 Z^1 + \dots + l_{\kappa m} Z^{\kappa m}. \quad (2)$$

From the relations

$$(\lambda_{-m} Z^{-m} + \dots + \lambda_m Z^m)^\kappa = l_{-\kappa m} Z^{-\kappa m} + \dots + l_{\kappa m} Z^{\kappa m}$$

we have

$$\kappa \log (\lambda_{-m} Z^{-m} + \dots + \lambda_m Z^m) = \log (l_{-\kappa m} Z^{-\kappa m} + \dots + l_{\kappa m} Z^{\kappa m})$$

which holds good for all values of  $Z$ . By differentiation with respect to  $Z$  and then clearing of fractions it becomes

$$\begin{aligned} \kappa (-m \lambda_{-m} Z^{-m-1} \dots + m \lambda_m Z^{m-1}) (l_{-\kappa m} Z^{-\kappa m} + \dots + l_{\kappa m} Z^{\kappa m}) = \\ (\lambda_{-m} Z^{-m} + \dots + \lambda_m Z^m) (-\kappa m l_{-\kappa m} Z^{-\kappa m-1} \dots + \kappa m l_{\kappa m} Z^{\kappa m-1}). \end{aligned} \quad (3)$$

Forming the coefficient of  $Z^{i-1}$  in the polynomial product, and remembering also that the rank of the middle  $l$  of this group reckoned from  $l_0$  is  $i$ , we get, by equating the two to each other by the principle of undetermined coefficients,

$$\kappa (-m \lambda_{-m} l_{i+m} - \dots + m \lambda_m l_{i-m}) = (i+m) \lambda_{-m} l_{i+m} + \dots + (i-m) \lambda_m l_{i-m}.$$

In the second member, let that part which does not have the coefficient  $i$  be transferred to the first member, then

$$-m \lambda_{-m} l_{i+m} - \dots + m \lambda_m l_{i-m} = \frac{i}{\kappa + 1} (\lambda_{-m} l_{i+m} + \dots + \lambda_m l_{i-m}). \quad (4)$$

Clearly then any coefficient  $l_i$  in the expansion, and the  $2m$  coefficients nearest to it, will be connected by the relation

$$\begin{aligned} (\lambda_1 l_{i-1} - \lambda_{-1} l_{i+1}) + 2(\lambda_2 l_{i-2} - \lambda_{-2} l_{i+2}) + \dots + m(\lambda_m l_{i-m} - \lambda_{-m} l_{i+m}) = \frac{i}{\kappa + 1} \\ \lambda_0 l_i + (\lambda_1 l_{i-1} + \lambda_{-1} l_{i+1}) + (\lambda_2 l_{i-2} + \lambda_{-2} l_{i+2}) + \dots + (\lambda_m l_{i-m} + \lambda_{-m} l_{i+m}). \end{aligned} \quad (5)$$

This is the fundamental principle of DeForest's analysis in his numerous interesting studies on the theory of probability. Let

$l_{i+1}$ ,  $l_{i-1}$ , etc., in (5) be expressed in terms of  $l_i$  and their differences. For this DeForest refers to a convenient formula given by Lacroix (Cal. diff. et intég., Paris, 1819) as follows:

$$l_{i+n} = l_i + \frac{n}{1} \Delta_1 + \frac{n^2}{2!} \Delta_2 + \frac{n(n^2-1^2)}{3!} \Delta_3 + \frac{n^2(n^2-1^2)}{4!} \Delta_4 + \frac{n(n^2-1^2)(n^2-2^2)}{5!} \Delta_5 + \text{etc.} \quad (6)$$

For brevity let us write also

$$\begin{aligned} b_0 &= \lambda_0 + (\lambda_1 + \lambda_{-1}) + (\lambda_2 + \lambda_{-2}) + \dots + (\lambda_m + \lambda_{-m}) \\ b_1 &= 1(\lambda_1 - \lambda_{-1}) + 2(\lambda_2 - \lambda_{-2}) + \dots + m(\lambda_m - \lambda_{-m}) \\ b_2 &= 1^2(\lambda_1 + \lambda_{-1}) + 2^2(\lambda_2 + \lambda_{-2}) + \dots + m^2(\lambda_m + \lambda_{-m}) \\ b_3 &= 1^3(\lambda_1 - \lambda_{-1}) + 2^3(\lambda_2 - \lambda_{-2}) + \dots + m^3(\lambda_m - \lambda_{-m}) \end{aligned} \quad (7)$$

etc., etc.

Denoting the numerator and denominator in the first member of (5) by  $N$  and  $D$  respectively, we get

$$\begin{aligned} N &= b_1 l_i - b_2 \Delta_1 + \frac{1}{2} b_3 \Delta_2 - \frac{1}{3!} (b_4 - b_2) \Delta_3 + \frac{1}{4!} (b_5 - b_3) \Delta_4 \\ &\quad - \frac{1}{5!} (b_6 - 5b_4 + 4b_2) \Delta_5 + \frac{1}{6!} (b_7 - 5b_5 + 4b_3) \Delta_6 \\ &\quad - \frac{1}{7!} (b_8 - 8b_6 + 19b_4 - 12b_2) \Delta_7 + \dots \\ D &= b_0 l_i - b_1 \Delta_1 + \frac{1}{2} b_2 \Delta_2 - \frac{1}{3!} (b_3 - b_1) \Delta_3 + \frac{1}{4!} (b_4 - b_2) \Delta_4 \\ &\quad - \frac{1}{5!} (b_5 - 5b_3 + 4b_1) \Delta_5 + \frac{1}{6!} (b_6 - 5b_4 + 4b_2) \Delta_6 \\ &\quad - \frac{1}{7!} (b_7 - 8b_5 + 19b_3 - 12b_1) \Delta_7 + \dots \end{aligned} \quad (8)$$

$$\text{or } \frac{N}{D} = \frac{i}{\kappa + 1}$$

When  $\kappa$  becomes infinite, and the successive values of  $l$  are regarded as consecutive ordinates to a limiting curve, we have

$$l_i = y \quad \Delta_1 = dy \quad \Delta_2 = d^2y \quad \Delta_3 = d^3y, \text{ etc.,}$$

and at the same time when the ordinates are set close together, the abscissa  $x$  corresponding to any  $y$  is  $x = idx$ . Thus (8) becomes the differential equation of the curve, and  $b_0, b_1, b_2$ , etc., are constants, and in fact are the successive moments of the area bounded by the curve and the axis of abscissas, these moments being taken about a vertical axis. Since any given polynomial may be reduced to one in which  $\Sigma(\lambda) = 1$ , by dividing it throughout by the sum of its coefficients, we therefore consider  $b_0 = 1$ . If a constant number is added to or subtracted from all the exponents of  $z$  in (1), it will not alter the value of  $l$  in (2). Hence by making  $Z^0$  the abscissa of the center of gravity,  $b_1$  becomes zero. Then any constant  $b_n$  in (7) will denote the sum of the products formed by multiplying each  $\lambda$  into the  $n$ th power of its abscissa reckoned from the new origin, if the common interval  $\Delta x$  between the abscissa is regarded as unity. With the above transformations, we may now write (8) in the following forms:

$$\frac{b_2 dy - \frac{1}{2} b_3 d^2 y + \frac{1}{6} (b_4 - b_2) d^3 y - \text{etc.}}{y + \frac{1}{2} b_2 d^2 y - \frac{1}{6} b_3 d^3 y + \text{etc.}} = \frac{-x}{(\kappa + 1) dx} \quad (9)$$

In the denominator of the first member let  $d^2 y, d^3 y$ , etc., be neglected in comparison with  $y$  and in the numerator let  $d^2 y, d^3 y$ , etc., be neglected in comparison with  $dy$ . Since  $\kappa$  is infinitely large, we may write  $\kappa$  instead of  $\kappa + 1$ .

Therefore

$$\frac{dy - \frac{1}{2} (b_3 \div b_2) d^2 y}{y} = \frac{-x}{\kappa b_2 dx}$$

Invert both members of this equation, subtract  $\frac{1}{2} (b_3 \div b_2)$  from each and invert them both back again. This gives

$$\frac{dy - \frac{1}{2} (b_3 \div b_2) d^2 y}{y - \frac{1}{2} (b_3 \div b_2) dy + \frac{1}{4} (b_3 \div b_2)^2 d^2 y} = \frac{-x}{\kappa b_2 dx + \frac{1}{2} (b_3 \div b_2) x} \quad (10)$$

Thus far we have carried on our treatment on the assumption that the origin of  $Z^0$  in the expansion is located at the center of gravity for the coefficient  $l$  in (2), which became the ordinate  $y$

to the limiting curve. Now in (10) let the origin be transferred from the center of gravity to another convenient point by putting

$$x - \frac{2\kappa b_2^2 dx}{b^3} \quad (11)$$

in place of  $x$ . This gives

$$\frac{dy - \frac{1}{2}(b_3 \div b_2)d^2y}{y - \frac{1}{2}(b_3 \div b_2)dy} = \frac{4\kappa b_2 dx - 2(b_3 \div b_2)x}{(b_3 \div b_2)^2 x} \quad (12)$$

In the first member, the numerator is the differential of the denominator. Without any further change of origin, we can write approximately as follows:

$$y = y + \frac{1}{2}(b_3 \div b_2)dy, \quad x = x + \frac{1}{2}(b_3 \div b_2)dx$$

Neglecting  $d^2y$  in the numerator and  $d^2y$  in the denominator, we get

$$\frac{dy}{y} = \frac{4\kappa b_2 dx - (b_3 \div b_2)^2 dx - 2(b_3 \div b_2)x}{(b_3 \div b_2)^2 [x + \frac{1}{2}(b_3 \div b_2)dx]}$$

Since the denominator  $y$  in the first member is supposed to be infinitely greater than the numerator  $dy$ , the denominator in the second member must be infinitely greater than its numerator, so that in the denominator we may neglect  $dx$  in comparison with  $x$ . Further let the constants be expressed by means of the two new constants

$$a = \frac{2b_2(dx)^2}{b_3(dx)^3}, \quad b = \kappa b_2(dx)^2. \quad (13)$$

Since  $\kappa$  is supposed to be an infinity of the second order,  $b$  represents a finite area. The equation will now stand

$$\frac{dy}{y} = \frac{dx}{x}(a^2b - 1) - adx, \quad (14)$$

and integration gives

$$\begin{aligned} \log y &= (a^2b - 1)\log x - ax + \log C \\ \therefore y &= Cx^{a^2b-1} e^{-ax} \end{aligned} \quad (15)$$

It now remains to determine the constant  $C$  in (15). Since

$\Sigma(\lambda) = 1$  in the given polynomial and  $\Sigma(l) = 1$  in its expansion, we shall have  $\Sigma(y) = 1$  in the formula (15). The  $y$  which DeForest uses, represents an elementary area, so that it should be understood to mean  $ydx$  in modern notation. Thus equation (18), omitting  $dx$ , gives the equation of the curve. Thus we have in DeForest's notation:

$$\frac{1}{dx} \int_0^\infty y dx = 1 \therefore \frac{C}{a^{a^b}} \int_0^\infty (ax)^{a^b-1} e^{-ax} d(ax) = 1,$$

which gives at once the value of  $C$  and we have

$$y = \frac{adx}{\Gamma(a^b)} (ax)^{a^b-1} e^{-ax} \quad (16)$$

the complete equation of the curve sought.

If we now transfer the origin of coördinates to the center of gravity by putting  $x + \frac{2\kappa b^2 dx}{b^3}$  in (11) or  $x + ab$  in place of  $x$  in (16), we have

$$y = \frac{dx}{ab\Gamma(a^b)} \left(\frac{a^2b}{e}\right)^{a^b} \left(1 + \frac{x}{ab}\right)^{a^b-1} e^{-ax} \quad (17)$$

Applying a known formula for  $\Gamma(n)$

$$\Gamma(n) = \left(\frac{n}{e}\right)^n \sqrt{2\pi} \left(1 + \frac{1}{12n} + \frac{1}{288n^2} + \text{etc.}\right)$$

(17) is reduced to

$$y = \frac{dx}{\kappa \sqrt{2\pi} b} \left(1 + \frac{x}{ab}\right)^{a^b-1} e^{-ax} \quad (18)$$

$$\text{where } \kappa = 1 + \frac{1}{12a^2b} + \frac{1}{288(a^2b)^2} + \text{etc.}$$

Returning to the meaning of the constants,  $a$  in (13) may be written

$$a = 2 \left( \frac{b_2 (dx)^2}{b^3 (dx)^3} \right) = 2 \left( \frac{\kappa b_2 (dx)^2}{\kappa b_3 (dx)^3} \right) \quad (19)$$

This shows that the part within the parenthesis may be regarded as the square of the quadratic radius divided by the cube of the

cubic radius, either in the first power of the polynomials or in its expansion to the  $\kappa$  power.

The value of  $a$  and  $b$  may thus be expressed by means of the coefficients  $\lambda$  in the given polynomial, or by means of the ordinates  $y$  to the limiting curve. When the  $\lambda$ 's and  $y$ 's are all positive  $\kappa b_2(dx)^2$  is the square of the quadratic mean error " $\epsilon$ " and  $\kappa b_3(dx)^3$  is the cube of what DeForest calls the cubic mean inequality " $\zeta$ ."

The constants in (13) will then be

$$a = 2\epsilon^2 \div \zeta^3$$

$$b = \epsilon^2$$

It will be seen then that the constants  $\epsilon^2$  and  $\zeta^3$  are respectively the second and third moments of Pearson and therefore can be advantageously determined by his method. The above sketch should enable the reader to get an idea of the method of DeForest's analysis, and this was my object in presenting it. The properties of the formula as well as the method of transformation of the present formula to the normal probability form are adequately treated in the original paper of DeForest. However, regarding these points, the reader will get still better information from Pearson's discussion on his curve of Type III.

Although I have not given the process of transformation of the formula to the normal form, DeForest's statement in this connection will be worth noting. He states that he would have obtained the normal form directly from the equation (9) if he had neglected  $d^2y$ . If instead of retaining only  $dy$  and  $d^2y$  he should also retain  $d^3y$ , the resulting equation, provided such is integrable, would doubtless give a limiting curve of a still more general form, of which the curve derived from (18) is but a particular case. Thus he thought that the probability curve and his curve (18) are only the first and second approximations to the actual form of an expansion to a high power.

From the foregoing discussion the reader will notice a close similarity between DeForest's formula, and Pearson's formula for the curve of Type III. For convenience, I shall enumerate some of the similar properties in these two curves.

- (1) Both are the skew binomial curves.
- (2) The curve is limited on one side the mean.
- (3) The analytical constants are determined from the first three moments.
- (4) Both can be reduced to the normal form.
- (5) Each is a particular case of a more general formula.

It will be demonstrated in the following pages that although these two formulas show no more apparent similarity yet the formulas are identical:

From the differential equation 
$$\frac{1}{y} \frac{dy}{dx} = - \frac{x + \frac{\mu_1}{2\mu_2}}{\mu_2 + \frac{\mu_1}{2\mu_2} x}$$

Professor Pearson obtained his formula for the curve of Type III which is usually written in the following form:

$$y = \frac{\alpha}{a} \cdot \frac{p^{p+1}}{e^p \Gamma(p+1)} \left(1 + \frac{x}{a}\right)^{\nu a} e^{-\nu x} \quad (20)$$

The following relations are also given

$$\mu_2 = \frac{p+1}{\nu^2}; \nu = \frac{2\mu_1}{\mu_2}; p = \frac{4\mu_1^2}{\mu_2^2} - 1; a = \frac{p}{\nu}.$$

Since the distance of the centroid vertical from the axis of  $y$  or maximum ordinate is  $\frac{1}{2} \frac{\mu_3}{\mu_2}$ , by changing the value of  $x$ , that is, putting

$$x = x + \frac{1}{2} \frac{\mu_3}{\mu_2}$$

(20) is reduced into the following

$$\begin{aligned} y &= \frac{\alpha}{a} \cdot \frac{p^{p+1}}{e^p \Gamma(p+1)} \left(1 + \frac{\frac{1}{2} \frac{\mu_3}{\mu_2} + x}{\frac{\mu_1}{2\mu_2} - \frac{\mu_3}{2\mu_2}}\right)^{\frac{4\mu_1^2}{\mu_2^2} - 1} e^{-\frac{2\mu_1}{\mu_2} \left(x + \frac{\mu_1}{2\mu_2}\right)} \\ &= \frac{\alpha}{a} \cdot \frac{p^{p+1} e^{-(p+1)}}{\Gamma(p+1) \left(1 - \frac{\mu_1^2}{4\mu_2^2}\right)^p} \left(1 + \frac{x}{2\mu_2 \div \mu_3}\right)^{\frac{4\mu_1^2}{\mu_2^2} - 1} e^{-\frac{2\mu_1}{\mu_2} x} \end{aligned}$$

$$\begin{aligned}
 &= \frac{\alpha}{a\sqrt{2\pi\mu_2}} \frac{\sqrt{2\pi\mu_1} p^{p+1} e^{-(p+1)}}{\Gamma(p+1)} \left(1 + \frac{x}{2\mu_1 \div \mu_2}\right)^{\frac{4\mu_1^2}{\mu_1^2} - 1} e^{-\frac{2\mu_2}{\mu_3} x} \\
 &= \frac{\alpha}{a\sqrt{2\pi\mu_2}} \frac{\sqrt{2\pi(p+1)} a p^p e^{-(p+1)}}{\Gamma(p+1) (p+1)^p} \left(1 + \frac{x}{2\mu_1 \div \mu_3}\right)^{\frac{4\mu_1^2}{\mu_1^2} - 1} e^{-\frac{2\mu_2}{\mu_3} x}
 \end{aligned}$$

and finally, as the result of transferring the origin to the centroid vertical, we obtain

$$y = \frac{\alpha}{\sqrt{2\pi\mu_2}} \frac{\sqrt{2\pi(p+1)} e^{-(p+1)} (p+1)^p}{\Gamma(p+1)} \left(1 + \frac{x}{2\mu_1 \div \mu_3}\right)^{\frac{4\mu_1^2}{\mu_1^2} - 1} e^{-\frac{2\mu_2}{\mu_3} x} \quad (21)$$

If we now apply to the above (21) DeForest's notation, that is,

$$\mu_2 = b \text{ and } 2\mu_1 \div \mu_3 = a$$

we obtain at once

$$y = y_1 \left(1 + \frac{x}{ab}\right)^{a^2b-1} e^{-ax}$$

where

$$y_1 = \frac{\alpha}{\sqrt{2\pi\mu_2}} \frac{\sqrt{2\pi(p+1)} e^{-(p+1)} (p+1)^p}{\Gamma(p+1)}$$

It only remains to see whether or not  $y_1$  in Pearson's formula is identical with DeForest's  $C$ .

We have

$$\begin{aligned}
 y_1 &= \frac{\alpha}{\sqrt{2\pi b}} \frac{\sqrt{2\pi(a^2b)} e^{-a^2b} a^2b^{a^2b-1}}{\Gamma(a^2b)} \\
 &= \frac{\alpha}{\Gamma(a^2b)} a e^{-a^2b} a^2b^{a^2b-1}
 \end{aligned}$$

Using the approximation formula for  $\Gamma(n)$  which DeForest uses (18) we have

$$y_1 = \frac{\alpha e^{-a^2b} a^3b^{a^2b-1}}{\left(\frac{a^2b}{e}\right)^{a^2b} \sqrt{\left(\frac{2\pi}{a^2b}\right)^{\frac{1}{2}}}}$$



$$= \frac{\alpha}{\kappa \sqrt{2\pi}} \cdot \frac{a \sqrt{a^2 b}}{a^2 b} = \frac{\alpha}{\kappa \sqrt{2\pi b}}$$

$$\text{where } \kappa = 1 + \frac{1}{12a^2b} + \frac{1}{288(a^2b)^2} + \text{etc.}$$

Since  $\alpha$  is unity in DeForest's formula, thus Pearson's formula for the curve of Type III immediately reduces to DeForest's. That is

$$y = \frac{1}{\kappa \sqrt{2\pi b}} \left(1 + \frac{x}{ab}\right)^{a^2b-1} e^{-ax}.$$

Thus DeForest's formula presents several interesting points which I herewith enumerate as the conclusion of the present report.

(1) DeForest's investigation gives an additional proof for the theoretical basis of Pearson's generalized probability curve.

(2) DeForest's investigation is interesting from an historical standpoint since he actually obtained one of Pearson's curves many years ago, and his work suggests a more generalized curve.

(3) Since DeForest's formula (see (18), p. 286) retains an elementary character, the curve fitting can be accomplished with comparatively small labor, and it can advantageously be used in place of the formula of Pearson for the curve of Type III.

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# ON THE OCCURRENCE OF PULMONARY ARTERIES ARISING FROM THE THORACIC AORTA

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WITH ONE FIGURE

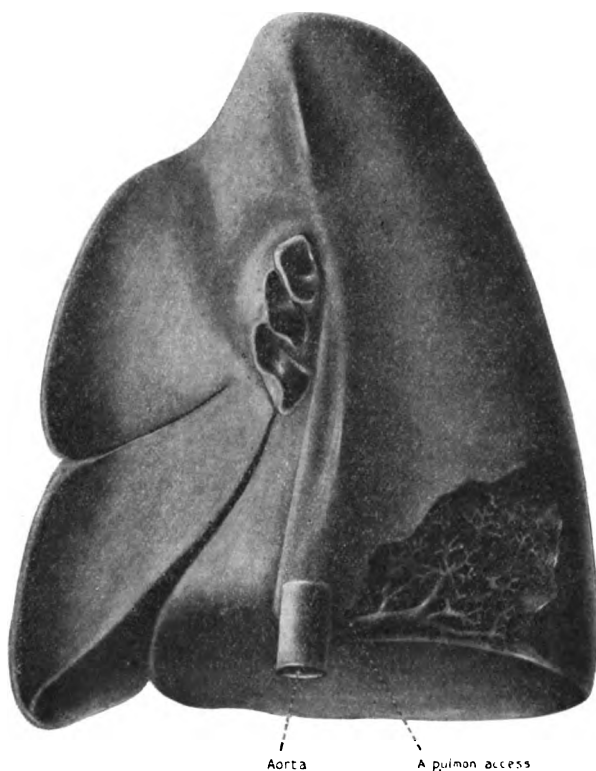
In the light of the recent advances that have been made in our knowledge concerning the early stages in the development of the vascular system, vascular anomalies take on a new interest. It is with this in mind that the writer reports the following apparently rare case of an accessory pulmonary artery arising from the lower part of the thoracic aorta.

The present case was observed in the anatomical laboratory of the University of Michigan. It occurred in a well nourished male white subject of medium height and build, aged 65 years. The cause of death was recorded as "heart disease." Upon dissection of the body the following conditions were found. From the front of the thoracic aorta, on a level with the tenth thoracic vertebra, 7 cm. above the cœliac axis, there was given off an artery, 7 mm. in diameter, which passed upward and to the right between the folds of the ligamentum latum pulmonis to the lower inner margin of the right lung. Here it entered the substance of the lung and broke up into branches which ramified among the lobules of the lower lobe, as is shown in the accompanying figure. There was no vein accompanying the artery. The lung itself otherwise appeared normal. From the aorta there were given off the usual number of intercostal arteries. Aside from the presence of the accessory pulmonary artery the pleura and structures in the mediastinum appeared entirely normal.

On reviewing the literature we have found nine cases recorded of accessory pulmonary arteries, in seven of which the accessory arteries arose from the thoracic aorta, one was given off from the

abdominal aorta, and one from an intercostal artery. In four of them as in the present case, the lung was otherwise normal. In the remaining five cases the arteries supplied accessory lobes.

The first case was reported by Huber (1777), who found in a two-year-old female child a large trunk arising from the thoracic aorta on a level with the seventh thoracic vertebra, which went



to the lower lobe of the right lung, where it entered the lung substance along its lower margin. In its course it gave off branches to the œsophagus and bronchial glands. Maugars ('02) described a case occurring in a seven-year-old child in which the abdominal aorta gave off an artery 5 mm. in diameter, which passed upward through the œsophageal opening in the diaphragm. After giv-

ing off branches to that muscle it divided into two trunks, one going to the lower lobe of each lung. Meckel ('20) described a case occurring in a nine-months-old child, where an artery, 9 mm. in diameter, was given off from the thoracic aorta about 1 cm. above its passage through the diaphragm. It passed upward and to the left to reach the lower border of the left lung, and divided into a medial and a lateral branch. The former was distributed to the lower portion of the lower lobe of the left lung. The lateral branch could not be followed. The vein that accompanied this artery terminated in the left pulmonary vein. Hyrtl ('39) recorded the occurrence in a new-born child of a pulmonary artery given off from the thoracic aorta supplying the left lower lobe, the left pulmonary artery proper supplied only the left upper lobe.

It will be seen that these four cases are essentially similar to our case. They differ only in that they were found in very young subjects. In one of them the artery supplied the right lung as in our case; in two of them it supplied the left lung, and in the remaining case it arose much lower down, below the diaphragm, and supplied both lungs.

In the following five cases the lungs were abnormal; Rektorzik ('61) described a case, observed in the body of a well-nourished girl who had died of peritonitis, of an accessory lobe 4 cm. long, 2½ cm. wide, and 1½ cm. thick. The lobe was situated between the left lung and the diaphragm. At the level of the tenth vertebra an artery 2 cm. long and having a diameter about the same as the left renal entered at the inner surface of the accessory lobe, where it divided into a number of branches. A single vein accompanied the artery and terminated in the hemiazygos. Rokitsansky ('61) found in the left pleural sac of a three-months-old child between the normal left lung and the diaphragm, an accessory lobe, conical in shape and containing no branches. Two arteries, which arose close to one another from the thoracic aorta on a level with the tenth intercostal space, entered the inferior surface of the accessory lobe. A single vein accompanied the artery and terminated in the vena azygos. Ruge ('78) described a case in a new-born child where an accessory lobe, situated between the left

lung and the diaphragm, received its blood supply by a small artery arising from the seventh intercostal. Humphrey ('85), during a postmortem examination on a year-old child, observed an accessory lobe between the base of the left lung and the diaphragm. A small pedicle which contained a small artery arising from the aorta and a vein which entered the hemiazygos connected the accessory lobe with the mediastinum. The most recent case was described by Simpson ('07), who found in a full-term foetus, situated below the right lung, an accessory lobe connected to the mediastinal space by a pedicle which contained an artery, the size of the internal carotid, which sprang from the aorta on a level with the tenth thoracic vertebra. In these cases of accessory pulmonary arteries connecting the systemic circulation with abnormal lungs one occurred on the right side and four on the left. Like the first four cases of normal lungs, they occurred in very young individuals.

Accessory pulmonary arteries have been described in certain vertebrates (amphibia and reptiles). Mudge ('98) described a case occurring in a frog where the caudal tip of the right lung was supplied by an artery arising from the coeliac artery. The caudal tip of the left lung was supplied by two arteries arising from the superior mesenteric artery. These arteries were accompanied by veins that terminated in the portal vein. A similar case has since then been described by Warren ('00). In the necturus there was reported by Williams ('09) an artery arising from the seventh intercostal artery, which reached the caudal tip of the left lung and passed to the cephalic extremity along the inner surface, giving off many branches to the lung substance in its course. According to Hyrtl ('37) and later confirmed by Calori ('42), accessory pulmonary arteries occur normally in ophidia. He observed a series of arteries arising from the aorta which passed laterally to the posterior vesicular portion of the much elongated lung.

For an explanation of vascular anomalies of this character we must undoubtedly look to the developmental factors involved. It is now generally believed, owing to the researches of Thoma and more recently of Evans, that the blood vascular system begins

as a capillary plexus (*area vasculosa*) which spreads in all directions. Subsequently channels develop through the capillary net which enlarge and become arteries and veins, according to whether the channel develops on the arterial or venous side of the extending plexus. Many of the capillary connections between the main channels finally disappear.

According to Flint and Evans, who worked on pig embryos, a plexus is formed which extends caudad from the developing pulmonary arches and envelopes the lung anlage in a rich capillary net. It is supposed that the pulmonary arteries are normally formed as channels in this plexus. In cases, however, like those we have been considering, where the pulmonary artery arises from the thoracic aorta, we must conceive of a plexus extending laterally from the primitive aorta and joining the pulmonary capillary plexus mentioned above, resembling the capillary net that extends laterally from the developing aorta to the limb buds as described by Evans. It is probable that we have to do with one of two conditions; firstly, it may be that a lateral primary capillary connection between the lung anlage and aorta is always present, and that this usually atrophies with the disappearance of the vascular connection between the lung and aorta. Occasionally a permanent channel is developed through it, and then we have the rare condition present which we have just reported. Secondly, it is possible that only occasionally a capillary plexus is laid down between the aorta and lung anlage, resulting in the production of a permanent channel constituting an accessory pulmonary artery. The frequent occurrence of small arteries extending laterally from the aorta between the folds of the *ligamentum latum pulmonis* to the lung, as described by Turner, and which I have frequently confirmed in this laboratory, support the former view. But we cannot expect a complete explanation of these anomalies until the detailed development of the pulmonary arteries has been worked out.

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## A SPECIMEN OF ANNULAR PANCREAS

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WITH TWO FIGURES

Among a series of 105 specimens of adult human pancreas and duodena recently studied in the anatomical laboratory at Cornell University, Ithaca, N. Y., one specimen of annular pancreas was discovered. This rare and interesting anomaly, in which the duodenum is encompassed by a ring of pancreatic tissue, I have been able to find reported but eight times in the literature. A compilation of the reports upon these specimens is herewith presented.

In Ecker's specimen, removed from the body of a young man, a narrow band of uninterrupted pancreatic tissue was found completely encompassing the descending portion of the duodenum. A duct tributary to the main duct, beginning in the ventral portion of the head of the gland in the neighborhood of the main duct, coursed ventrodorsally through the ring of pancreatic tissue, received numerous tributaries from the ring and head and terminated dorsally by emptying into the main pancreatic duct.

Judging from the plate accompanying the article, the duodenum was constricted at the level of the ring and dilated immediately cephalic to it. No mention was made of the condition of the stomach in this case.

In Auberg's case cited by Ancelet, the terminal portion of the duodenum was imbedded in pancreatic tissue, the description giving the impression that there had been a great development of the head of the pancreas. There was some narrowing of the duodenum, but no mention was made of any change in the stomach.

J. Symington describes a case in which two processes of the pancreas passed from the cephalic part of the head of the gland towards the right side, one ventral and the other dorsal to the duodenum. They

blended on its lateral wall so as to form with the head of the gland a ring of pancreas encircling the duodenum. The processes became somewhat narrower as they passed laterally, and the portion of gland on the right side of the duodenum was about 12 mm. in vertical extent. The circumference of the duodenum where it was surrounded by pancreas was about 60 mm., while cephalic and caudal to that level it was more than three times as large. On dissecting out the ducts of the pancreas, nothing unusual was observed in their arrangement. The common bile duct opened into the duodenum caudal to the seat of constriction.

Anton Genersich was the next to describe a specimen. He says that he found in the body of a 37-year-old man, who had died of double pneumonia, the following condition of the pancreas: The head of the pancreas was unusually large. It measured "6 cm. hoch, 7 cm. breit." A process of normal pancreatic tissue 3 cm. broad passed from the ventral surface of the head of the pancreas transversely across the ventral surface of the descending duodenum, 7 cm. from the pylorus. Turning around the right surface of the intestine, this process, reduced in size to 1.5 cm. breadth and 0.8 cm. thickness, traversed the dorsal surface of the duodenum, and with a breadth slightly augmented, fused with the dorsal surface of the head of the gland. The length of the ring was 4.5 cm. A duct coursed from the ventral limb of the ring through the dorsal limb to empty into the ductus pancreaticus. At the ring the intestine was narrowed to the diameter of a thumb (6 cm. circumference). The portion of duodenum cephalic to the ring was much dilated and had thickened walls. The stomach itself was noticeably dilated with a thickened muscularis. Caudal to the ring, the duodenum measured only 7 cm.

Louis Sandras reported a specimen taken from a man aged seventy years. A ring of pancreatic tissue 3.5 cm. broad surrounded the descending portion of the duodenum causing a slight constriction with a slight dilatation immediately cephalic to it.

Dr. Hugo Summa of St. Louis, Mo., exhibited a specimen of annular pancreas at the meeting of the American Medical Association at Atlantic City, N. J., 1900; Dr. J. S. Thacher of New York also presented a similar specimen in 1893. Descriptions of these specimens are not, however, at present available.

Tieken's specimen was from a man thirty-four years old. The caudal part of the descending duodenum was surrounded by a complete ring of pancreatic tissue. On the pancreatic side both ventrally and dorsally, this ring measured nearly 4 cm. broad, but narrowed to 2.75 cm. on the

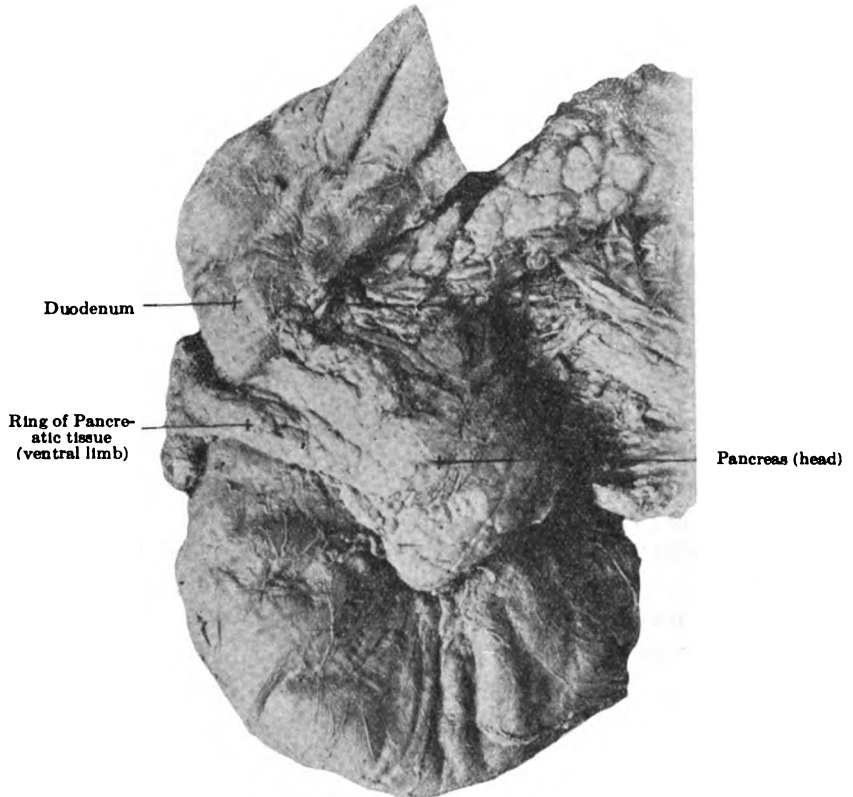
lateral side of the duodenum, where it was 1 cm. thick. This caused a constriction of the duodenum with sacculations of the intestine cephalic to the narrow portion, dilatation of the pylorus, hypertrophy of its walls, and hypertrophy of the walls of the stomach but no marked dilatation. The annular band was loosely adherent to the duodenum. The pancreatic duct opened in the usual way in common with the bile duct at the cephalic part of the ring. The vascular supply was not abnormal and sections from head, body, and annular portion showed normal pancreatic tissue.

In the Cornell specimen, there is nothing deserving of special mention in the head of the pancreas. The pancreatic duct courses through the dorsocaudal segment of the head of the gland, while the accessory duct occupies its usual position cephalic and ventral to it. The bile duct joins the main pancreatic duct as usual. Springing from the cephalic part of the ventral surface of the head of the pancreas, a narrow band of pancreatic tissue 2.0 mm. thick and 9.0 mm. broad passes transversely across the ventral surface of the pars descendens duodeni. As is shown in the photograph, this ring expands abruptly upon the lateral aspect of the duodenum, measuring 31.0 mm. in the cephalocaudal diameter. Thence it passes across the dorsal surface of the duodenum, beginning with a breadth of 17.0 mm. and expanding to 31.0 mm. at its junction with the cephalic portion of the dorsal surface of the head of the gland. The ring is thickest (7.0 mm.) at the lateral expansion and thinnest on the ventral surface of the duodenum (2.0 mm.). It is loosely affixed to the duodenal wall by means of connective tissue.

Ventrally in the head of the pancreas, at the level of the ring, there begins by minute radicles a duct in nowise connected with the accessory pancreatic duct. This duct courses with uniformly augmenting calibre through the middle of the substance of the ventral limb of the ring from left to right, thence, through the dorsal limb towards the head of the gland, where, passing dorsal to the common bile duct, it opens with a diameter of 3.0 mm. into the main pancreatic duct. This duct drains a small portion of the cephalic segment of the head of the gland and the whole of the ring.

The duodenum possesses, when flattened, an average diameter

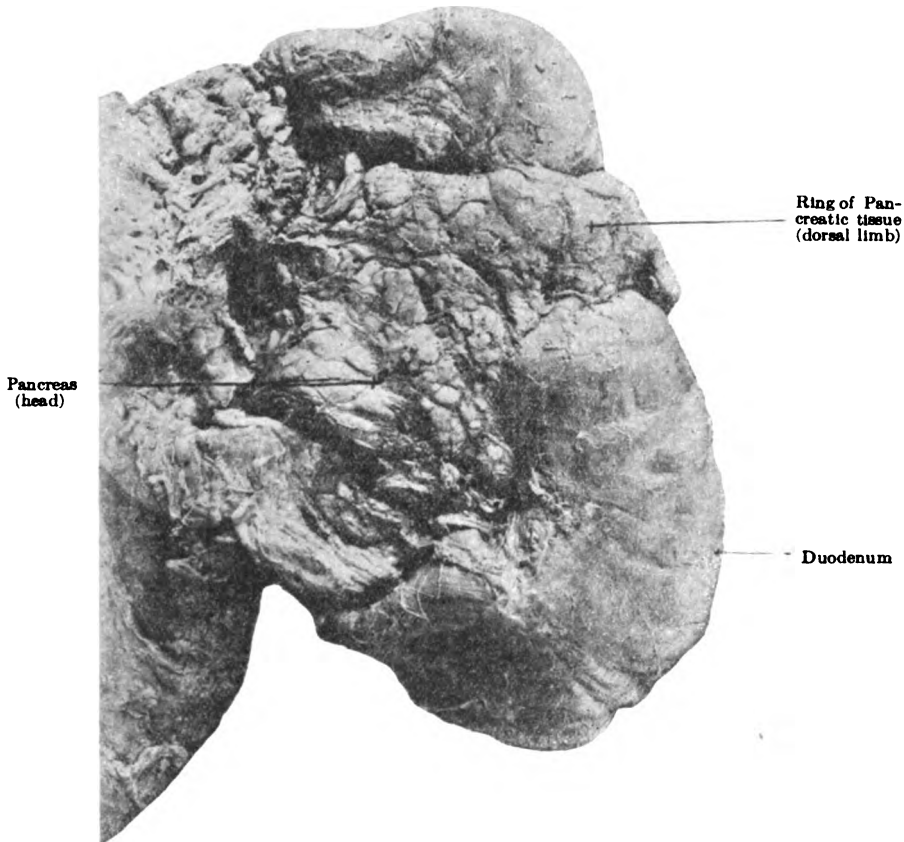
of 2.7 cm. immediately cephalic to the ring of pancreatic tissue, narrowing quickly to 2.0 cm. at the ring and dilating immediately caudal to 4.0 cm. There is no thickening of the duodenal wall either at the ring or cephalic to it. The stomach is neither dilated nor are its walls thickened.



Ventral Aspect

An explanation of the cause of this anomalous condition must be sought for in the embryology of the gland. Zimmermann, Felix, Jankelowitz, Helly, and Ingalls, among others, describe the pancreas as arising from the duodenal wall from two anlagen, one dorsal and one ventral. The ventral anlage consists of two

parts, left and right. Ordinarily the left half atrophies. The right half is carried around dorsal to the duodenum consequent to the growth and rotation of the duodenum with the stomach. Ultimately this anlage forms the caudal segment of the head of the



Dorsal Aspect

gland, fusing with the dorsal anlage. In this right half of the ventral anlage the terminal portion of the pancreatic duct is developed.

It is a significant fact that the specimens of annular pancreas which have been dissected show a duct traversing the ring and joining dorsally with the main pancreatic duct, not emptying

into the accessory duct. This seems to indicate that this ring of tissue is either a persistence of the left half of the ventral anlage or an excessive growth from the right half of the same anlage. If it is this latter case the excessive growth has taken place ventral to the duodenum and to the left at the time that the remainder of the same half was growing or being carried dorsally to ultimately fuse with the head of the gland.

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## PRACTICAL STATE BOARD EXAMINATIONS IN ANATOMY

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During the past twenty years the states of this Union have almost all passed laws which have for their purpose the restriction of the practice of medicine to properly qualified physicians. The determination of the qualifications is entrusted to State Boards of Medical Examiners. The qualifications are determined in part by the standards of the school from which the applicant has obtained his doctor's degree, in part by a special examination. Many states have reciprocal relations with other states, so that one licensed to practice in one state may obtain without further examination a license to practice medicine in another state with which the first has reciprocal relations.

The purpose of the laws regulating the practice of medicine is excellent. They have doubtless played some part in furthering the splendid advance in standards of medical education which has been taking place in this country during the last thirty years. In general, however, as devised and executed, they come very far from achieving their purpose of restricting medical practice to the properly qualified. This failure is due chiefly to methods used in choosing medical examiners, and to the methods used in conducting examinations.

The examiners are by law usually restricted to those who have no affiliations with any medical school, and as a rule the terms of appointment are comparatively short. Most of them, therefore, necessarily lack the experience in medical science, in teaching, and in examining, such as thoroughly to qualify them for testing the real ability of the candidates for license. Fortunately there are apt to be a few men on every State Board who through previous experience in teaching in a medical school, or through



natural ability and deep interest in medical science are able to keep the standards from falling as low as we might *a priori* expect. The first aim of those interested in maintaining by law high standards in the practice of medicine should be to see that the state laws are so amended as to permit the appointment of the most competent examiners possible, irrespective of affiliations with medical schools, and to arouse a public sentiment which would insist upon the appointment of men of this character. The public should be made to understand how seriously important it is to have high standards in medical practice; what irreparable harm may be done by incompetent and irresponsible practitioners. The public could well afford to pay liberally for the services of competent men to serve on the licensing boards. At present, if we may judge of public opinion from legislative action, the licensing of physicians is largely looked upon as a sort of trade-union scheme devised to restrict competition among physicians, and the public insists that various sorts of irregulars shall not be debarred from entering into this competition. The applicants for license pay the expenses of the licensing board. The broader public interests are lost sight of.

Without competent examiners it will do little good to change the methods of conducting examinations yet it is obvious that the methods at present in vogue are far from adequate to test the fitness of candidates to practice medicine. Answers to a series of examination questions on various subjects in medical science and medical practice are hurriedly written during sessions lasting a day or two; each examiner reads the answers to the series of questions which he has set and the candidate is given a license if in the opinion of the examiners he has answered correctly a sufficient number of the questions in a sufficient number of subjects. The test is one solely of the memory and to a less extent of the power of written expression. No test is made of the most important side of medical education—the training in scientific, chemical, physical and biological methods and in the application of these methods in medical practice. By restricting candidates to graduates of medical schools coming up to a certain standard some training in method is assured. As a rule, however, these standards are highly artificial and relate to external rather than

internal conditions; to requirements for admission and to number of years of medical study demanded rather than to facilities, methods of teaching, and teachers. If medical schools could all be trusted, the doctor's degree would be all that should be necessary to entitle a man to practice. If they cannot be trusted, a test of a candidate's ability to use scientific methods and to apply these in medicine is absolutely essential if we are to insist that only the properly qualified are to practice medicine.<sup>1</sup>

The time has come, I think, for medical teachers to insist upon practical examinations given by competent examiners. If the teachers in each of the fundamental branches of medical education will do their best to bring this condition about I believe that much can be accomplished.

In order to affect a reform one should have definite ideas about practical improvements. As anatomists, we should, I think, consider carefully such changes in methods of conducting state examinations in anatomy as will best test the candidate's ability to think anatomically and to use anatomical methods in medical practice. With this in mind I suggest the following:

*Gross anatomy.* The Board of Medical Examiners should have a considerable number of sets of frozen sections cut in various planes through the body and some special preparations of various more important regions and organs, including skeletal preparations, the brain, and spinal cord, organs of special sense, larynx, heart, lungs, abdominal and pelvic viscera. These specimens could be kept for years. From time to time new specimens could be added and worn specimens discarded. At the time of holding the examination the specimens should be displayed in a special room set apart for that purpose. The candidates in small groups should be led about from preparation to preparation and thoroughly quizzed; or, if it seemed preferable, each candidate could be given a certain number of preparations carefully to describe in writing.

Where an abundance of human anatomical material could be obtained it would probably be well to require, in addition to the practical examination outlined above, a dissection of some small

<sup>1</sup> In some states, such as Ohio and Minnesota, a successful beginning along the line of practical examinations has already been made.

region of the body. In case human anatomical material were not abundant, dissection of a part of some mammal might be required.

*Microscopical anatomy.* The State Board of Medical Examiners should have a good collection of microscopical preparations of various organs and tissues, including sections through the central nervous system and some embryological specimens. This collection, like that in gross anatomy, could be added to from year to year and damaged specimens removed. It would probably in most cases be practical to hold the examination in a university town so that microscopes could be borrowed for the examination, if it should at first seem too great an expense for the State Board to purchase a sufficient number. Indeed it might be well to require every candidate to come to the examination supplied with a good microscope, since every properly equipped physician should have one. Each member of the group of candidates examined should be given a few prepared specimens to examine. He should give a description of these. In addition, he should be required to prepare specimens for study so that his ability to section, tease out and stain specimens could be tested. For sectioning freezing microtomes might be used.

Against the immediate introduction of examinations of the character outlined above may be urged the extra time and the extra number of examiners needed as compared with the methods at present in vogue. By properly subdividing the whole number of candidates into small groups and examining each group at any given time in a different subject, the time for conducting the whole examination would not need to be very greatly increased since each group might within two or three days be given a fairly good practical examination in each of the chief medical subjects. At present the Boards of Medical Examiners are, as a rule, so large that the asking of questions for each of the chief subjects is entrusted to one or two men. These men, if competent, by taking successive small groups of candidates for a few hours, could within a few days, get a far better idea of the training and ability of those candidates than is at present possible.

## BOOK REVIEW

QUAIN'S ELEMENTS OF ANATOMY, Eleventh Edition, Vol. 3, Part 2. E. A. Schäfer and J. Symington. Containing the descriptive anatomy of the peripheral nerves and of the organs of special sense. Longmans, Green and Company, London, 1909.

The second part of the third volume of Quain's Elements of Anatomy, eleventh edition contains the descriptive anatomy of the peripheral nervous system and of the organs of special sense. This part which completes the section of neurology has been edited and to a large extent rewritten by Professors Schäfer and Symington, and incorporates the results of their investigations along these lines. It will thus be readily understood that this volume dealt with by such capable hands is a valuable contribution to anatomical literature.

The part dealing with the special senses is particularly good and is the most complete anatomical description in English of the sense organs. The minuteness of the description will be apparent when it is noted that nearly one hundred pages are given to the anatomy of the eye and nearly seventy to the anatomy of the ear. The illustrations are numerous and extremely well chosen, some from Schäfer's work and a considerable number from the very important papers of Symington. Especially worthy of commendation are those of Symington dealing with the temporal bone at various ages. The most important literature up to the time of the publication has been referred to and often incorporated in the text. To the advanced student this part will prove an exhaustive and a most readable account. The practitioner and the specialist will find it a valuable addition to their library; they will be particularly pleased with the fact that many of the anatomical data are so graphically and suggestively stated as at once to emphasize their diagnostic and surgical importance.

On reading the part on the sympathetic nervous system, one is at times delighted with the description, at other times disappointed with the meagerness of the details. It is difficult to account for such inequality in this English text-book, coming as it does from schools where so much has been done if not directly on the anatomical side at any rate on the anatomical physiological side to elucidate this part of the nervous system. To select but one section which appears disappointing one might point to the description of the cardiac plexus. This account is virtually the same as in the addition of 1903 in spite of much work that has been done since then. For instance one might well question whether the description given of the nerves in the ventricle as being deficient in ganglion cells represents the present state of our knowledge after the many papers which have been published in recent years.

The chapter on the peripheral nerves has many alterations which enhance the value of the work. In no part more than that dealing with the peripheral nervous system has Quain's *Anatomy* come to be regarded not as a text-book of anatomy, but more as a work of reference. It is here that one realizes how further additions would have added to the value of the description, and one regrets at times a lack of detail. To particularize, it might be urged that in view of the importance of the ganglia in connection with the cranial nerves, a more detailed account might have been given and not simply the old account reinserted. The description of these ganglia has been an outstanding feature in Quain's *Anatomy*, and one would have liked to have seen this part rewritten. For instance, the account of the sphenopalatine ganglion might have been improved were there more clearly stated the relation or non-relation to it of the sphenopalatine nerves, an alteration which might be further emphasized by the addition of a new dissection to illustrate the point. A little more information in regard to the geniculate ganglion would not have been amiss; would it have been too much to ask some more information in regard to the fibers in the chorda tympani nerve and their relation to this ganglion?

These are after all but small blots. Most of the defects tend rather along the line of omission than commission; to remedy this might have transgressed the limits allowed by the publisher. Of recent years we have had a surfeit of new anatomical text-books and of new editions. The time is rapidly approaching when we shall have an urgent demand for a reference book in English comparable to Poirier and Charpy and to Bardeleben. At present Quain approximates to this. It has never been the textbook of the ordinary medical student, but the source to which the advanced student turns for information. This it will still continue to be, and taken as a whole this volume will worthily keep up the traditions of that great anatomical work.

The carefully selected references to current literature given at the foot of the page instead of as formerly at the end of the chapter, the improved type and better paper in this edition are of decided advantage.

J. GORDON WILSON.

#### THREE RECENT TEXT-BOOKS ON TOPOGRAPHICAL ANATOMY

LEHRBUCH DER TOPOGRAPHISCHEN ANATOMIE. Prof. H. K. Corning (Basle). Second Edition, 1909. Wiesbaden: J. F. Bergmann.

TOPOGRAPHISCHE ANATOMIE. Prof. Oskar Schultz (Würzburg). Second Edition, 1909. Vol. I, Lehmann's med. Atlanten, Munich.

ATLAS AND TEXT-BOOK OF TOPOGRAPHIC AND APPLIED ANATOMIE, by Oskar Schultze, edited with additions by G. D. Stewart (Bellevue), 1905. Philadelphia and London: W. B. Sanders and Co.

The increase in the numbers of works on topographical, regional, applied and surgical anatomy is a fair criterion of the demand on the part

of practitioners and students of medicine for the detailed regional relations. Practically all of these works acknowledge this as their mission. The ideal topographical anatomy, like the ideal systematical anatomy, is a thing of the future, still it would be very strange indeed if the developments in the presentation of the subject did not show marked advance in the past ten years. Inasmuch as there is no text-book on anatomy into which a greater personal equation enters than in a regional treatise, it may be well to state in advance the reviewer's conception of what an ideal topographical anatomy should include and base the criticism of the volumes reported from this standpoint.

The ideal topographical anatomy should be a sort of reference work to which the physician may go to refresh his memory on the regional relations. The latter, however, are directly proportional to the frequency of variations in a given part, and no topographical treatise may be said to be complete without some consideration of the more important anomalies, particularly those due to defective development. In order to facilitate description it has become necessary to block out the body into given surface regions, but where the subdivisions become too complicated, or are carried into the deeper regions in too great a detail, confusion to a reader who has not made anatomy his vocation is imminent. The work should contain the best of illustrations drawn by artists gifted in anatomical presentation and at no time should the figures be so complicated or extended that the evident relations under discussion are in any way obscured. Many smaller figures, drawn especially to bring out certain few points, are better for this reason than the large and complicated full page illustrations. The text should be clear, if necessarily concise, and should conform to the figures in position, detail in relation and terminology. The introduction of frequent references to the classical works on a given region stimulates the student to a more thorough study of the problem before him—an essential requirement of a reference text-book. The terminology should be an accepted or preferably the accepted one; technical synonyms should be avoided; and the nomenclature adhered to even at the risk of unsightly typographical construction. If the function of the book is to be one of ready reference particular care should be exercised in the indexing that the reader may readily find all that is within the covers on a given topic. The introduction of the practical application of certain of the facts presented serves to lighten the mass and make the book more readable. Unfortunately, the surgeon best qualified to write upon this phase of the subject usually does so at the expense of important anatomical detail and the work takes the tone of an abbreviated text on surgical procedure.

The book by Corning is one of 772 pages with 653 illustrations of which 424 are in color (an increase of 57 pages and 49 figures over the first edition). The print is excellent and the volume is an example of plate reproduction, color work and bookmaker's art that might well be imitated by our American publishers. The paper is of durable quality and highly glossed to facilitate the plate process. The drawings are quite uniformly excellent and are for the most part by Mayer of Basel

and executed under Prof. Corning's personal direction. The illustrations are well chosen and limited to the region under discussion. Microtome sections have been introduced to replace some of the older schematic drawings of the hand, foot, eye and mouth regions. The nomenclature, which lacked uniformity in the first edition, has been reedited and made to conform to the B. N. A.

The chapter on the head (180 pages and 148 illustrations) has been rewritten in part to eliminate the misleading nomenclature which was particularly conspicuous in this chapter and a number of the inaccuracies in the plates have been corrected. The cranio-cerebral topography follows the accepted work of Froriep and Krönlein and additional illustrations have been added to show the internal cerebral topography, intentionally omitted from the first edition. The same may be said of the hypophysis, which now has two figures devoted to it and the description of the mouth cavity has been made more complete. The microtome sections of the eye and the nose are a decided advance over the old schematic drawings and are far more instructive. The topography and variations of the nasal sinuses is taken from Killian's classical monograph on this subject. A description of the common developmental defects about the mouth and nose regions would not be amiss. The chapter, on the whole, is much stronger than that of the first edition.

The neck region (65 pages and 47 figures) is particularly good in the treatment of the fascial compartments. Hitherto the fasciæ have been considered as active factors in the regional subdivisions rather than as passive factors which are dependent on the amount and character of movement in the surrounding structures for their development. Again Corning avoids the common fault of the surgical anatomies in over-schematizing these compartments. The larynx is well described both in structure and topography and illustrated in part by microtome sections. The parathyreoid bodies, omitted in the first edition, receive attention and six new figures have been added to emphasize their relations. The cervical ribs are briefly considered with one new figure to show their relation to the subclavian vessels and brachial plexus. The chapter might be said to suffer from an excellent fault in the over-detail of the boundaries in the regional subdivisions.

The thorax occupies 92 plates and 65 illustrations. The treatment of the chest wall is excellent and the schematic drawings illustrating the vascular supply, and in particular the lymph return from the mammary region are well chosen. The lung topography is based upon Merkel's work and the heart relations are satisfactorily considered. The lateral dissections of the mediastinal spaces on formalin hardened bodies are supplemented by the cross sections after Braune and the addition of the frontal and sagittal chest sections is welcomed.

The abdomen (157 pages and 139 illustrations) chapter has had added to it some 17 figures, which are for the most part devoted to the variations in the abdominal organs. The chapter is perhaps the most satisfying in the book, and pains and expense have not been spared to make this region quite complete both in text and in illustrations. It is grati-

fying to see the work of Americans recognized in one figure from Piersol; one from Mall and one from Broedel. It is rather curious that while abnormalities in the kidney form receive attention, no mention is made in this chapter of the not infrequent double ureter.

The chapter on the pelvis deservedly receives 100 pages and 100 illustrations. Four new figures have been introduced on the pregnant uterus and are welcome. A consideration of the congenital abnormalities would make the chapter more complete. The chapter on the back (9 pages and 9 figures) has had added an illustration of the suboccipital triangle and is confined for the most part to the spinal column and to the topography of the cord and its membranes. The structure of the cord and the relations of the tracts at different levels is omitted. The upper extremity (79 pages and 70 figures) and the lower extremity (77 pages and 69 figures) with the exception of a new figure of the axillary topography are much the same as in the first edition and are quite adequate. The projection drawings of the relation of the bones to the surface, the schematic presentation of perspective views of the fascial compartments and the selection of the cross and longitudinal are particularly well chosen. These two chapters contain a number of minor errors, which would seem unnecessary in a second edition, but Professor Corning was ill at the time the proof was read and had to rely on others for this most important work.

Taken as a whole, Corning's work appeals to the reader as the most complete and carefully illustrated volume on topographical anatomy that has appeared to date. The points that stand out in particular are the thorough work on the lymphatic system, the treatment of the body fascia, and the comprehensive chapters on the thorax, abdomen and pelvis. It is to be regretted that this book, like most of its German contemporaries, is poorly indexed. The work is decidedly worthy of translation into English, and if it can be published in anything like the style employed by Bergmann, would merit a widespread sale.

The book by Schultze represents the atlas type of topographical anatomy. The second edition has increased the number of illustrations by 115, making a total of 205 figures and 22 multichrome lithographic plates. The work is directed especially toward the medical student, and the text of the second edition has been modified so that the practical application of the various facts has been placed in separate paragraphs. Schultze believes this is desirable in that the student in preparing for the physicum may avoid the parts concerned in surgical application. The text is considerably abbreviated and no attempt is made toward the thorough discussion of any given region. The chapter on the head occupies 54 pages with 49 figures and 4 plates. The text, while concise, is uniquely clear and satisfying. The neck (17 pages, 20 figures and 1 plate) lends itself to criticism from the standpoint of an atlas in the selection of one figure to show the uncommon *Art. thyreoidea ima* and two figures showing the relations of the right subclavian artery, when it arises from the descending aortic arch to œsophagus and trachea. A



good figure of the parathyroid glands and of the common cervical ribs, particularly in their relation to the subclavian vessels and brachial plexus, would be a welcome substitution for the illustrations mentioned. The upper extremity (26 pages and 27 figures) has a figure of the opened axillary space that is quite inadequate; shows one diagrammatic cut of the brachial plexus, which is not only unnecessary, but is also somewhat incorrect; devotes two figures to the high bifurcation of the brachial artery and to the very uncommon high origin of the common interosseous artery. Fig. 94, showing the relatively infrequent superficial palmar arch formed in part by the Art. mediana, might be replaced to advantage by a drawing illustrating the projected relation of the carpal and metacarpal articulations and the superficial and deep arches to the surface. Other than this the illustrations and the text are good.

The chest (30 pages, 30 figures and 5 plates) and the abdomen and pelvis (56 pages, 54 figures and 12 plates) are the best in the book, although neither text nor plates are on a par with Corning's chapters on these parts. It is not the reviewer's opinion that the His models reproduce well. They are most valuable in their three dimension state. The lower extremity is fairly complete save in the consideration of the lymphatic system (20 pages and 20 figures).

As a whole the text is a trifle better than the illustrations, which is perhaps a little curious in an atlas. It will undoubtedly lend itself well to the needs of the medical student for "hitting the high places," but for extended study and for reference it gives too little in detail. The greatest point of criticism, perhaps, may be found in the rather confusing terminology which, however, is not serious, and in the poor indexing. The index, for example, gives Artic. sternoclavicularis, but hip, knee and shoulder are under the common names, while ankle and wrist are not mentioned at all. It would seem that for the German medical student, with the thorough training in Latin, the technical nomenclature would be quite sufficient. The plate illustrations are, if anything, a trifle too extended, and are not arranged to conform with the text. This book has merited translation into English and it is interesting to see what changes have been brought about in the translator's hands.

Stewart's edition of the book is little more than a literal translation of the text with a few surgical suggestions added. The plates have been made in Germany and are covered by a sheet of tissue paper on which has been printed the common names for the structures. In the reviewer's opinion this is not only unnecessary, but also serves to make the plates inaccessible. In addition to this Stewart has appended to each region a set of review questions chiefly concerned with the surgical facts and which would undoubtedly lend themselves to preparation for examination. The index has been decidedly revised and is about double the length in this book of that in the second German edition but the great parts of these terms are surgical and some of them curious—vein of anger, epipericardium, lymphatic glands in the subinguinal region, p. 158, or eleven lines farther down in the index, Lymphoglandulæ subinguinales, p. 158, etc.

The second edition of Schultze's book would lend itself better to translation because of the increased number of illustrations and because the editing of the surgical application could be done in just as complete a manner as the translator might desire.

A. G. POHLMAN.

# STANDARD SIZES FOR ILLUSTRATIONS

IN THE JOURNALS PUBLISHED BY

## THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

SUGGESTED BY PROFESSOR SIMON HENRY GAGE

AND APPROVED BY THE ADVISORY BOARD OF THE WISTAR INSTITUTE AT ITS LAST MEETING  
(APRIL 1910)

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It is desirable that a uniform system of magnifications should be followed in making illustrations for publication.

This system has been adopted: 1, 2½, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 75, 80, 90, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1250, 1500, 2000.

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# DIE MORPHOLOGIE DER BLUTZELLEN UND IHRE BEZIEHUNGEN ZU EINANDER<sup>1</sup>

VON

PROF. DR. FRANZ WEIDENREICH

*Strassburg*

MIT NEUNUNDSCHZIG FIGUREN

Bei allen Wirbeltieren, die wir kennen, lassen sich die im Blute kreisenden Zellen nach ihrer morphologischen Gesamterscheinung in zwei Hauptgruppen sondern, in die roten und in die weissen Blutkörperchen. Während aber die roten Blutkörperchen, die überall und ausschliesslich die Träger des Hämoglobins sind, sich bei jeder Art nur in einer einzigen charakteristischen Form finden, sind die weissen Blutkörperchen morphologisch und funktionell nicht gleich, sondern treten in verschiedenen Typen auf, die allerdings bei allen Arten, soweit die bisherigen Untersuchungen reichen, im grossen und ganzen wiederkehren. Diese Verschiedenheit der farblosen Blutelemente war schon Wharton Jones aufgefallen, aber die Fortschritte der Färbetechnik, die zuerst und systematisch von Ehrlich für das Blut angewandt und ausgebaut wurde, haben die Unterschiede deutlicher erkennen lehren. Es ist interessant, dass die gleiche Differenzierung der Blutelemente schon bei den Wirbellosen zu beobachten ist; wenn auch besondere Hämoglobinträger hier nur bei wenigen Arten vorkommen, so ist doch die morphologische Gliederung der weissen schon ziemlich weitgehend und prinzipiell mit der der Wirbeltiere übereinstimmend. Leider hat man auf diese durch die gesammte Tierwelt durchgehende Erscheinung viel zu wenig geachtet und ist damit ganz beträchtlich hinter Jones zurückgeblieben, der trotz der Mängel seiner Methodik schon auf

<sup>1</sup>Nach einem auf der 25. Tagung der American Association of Anatomists in Boston (29. Dez. 1909) erstatteten Referate.

dem Wege einer allgemeinen vergleichend-histologischen Analyse aller Blutzellenformen die einzelnen Typen in ihrer Bedeutung zu erfassen suchte. Erst in neuerer Zeit beginnt man sich wieder des Vorteils einer derartigen Vergleichung zu erinnern und so ist zu hoffen, dass durch die Verpflanzung des Blutzellen-Problems aus dem engen Bereich klinisch-pathologischer Rücksichten mit ihrer Bevorzugung des Menschen in das weite Gebiet allgemeiner vergleichender Histologie auch manche heute noch strittige Teilfrage ihrer Lösung näher geführt werden kann.

Die Grundlage solcher Untersuchungen bildet natürlich die genaue Kenntniss der morphologischen Besonderheiten der einzelnen Zelltypen, leider hat man lange Zeit viel zu viel Wert auf die färberische Qualität der granulären Plasmaeinlagerungen gelegt, die einige Leucocytenformen auszeichnen, und darüber andere wesentlichere Momente vernachlässigt. Dadurch dass man ferner das Hauptaugenmerk auf den Menschen konzentrierte und höchstens noch einige Säuger berücksichtigte, wurden die Besonderheiten der Blutelemente der übrigen Wirbeltiergruppen weniger bekannt, ein Umstand der für eine weitergehende Vergleichung erschwerend wirkt. Die gleiche Schwierigkeit ergibt sich aber auch für die Untersuchung der verschiedenen Blutzellenformen zu einander. Auch hier erweist sich die Beschränkung auf den Menschen oder einige Säugetiere als ein Fehler, da die Verhältnisse bei niederen Wirbeltieren zum grossen Teil viel einfacher liegen und auch leichter studierbar sind.

Die wenigsten Artverschiedenheiten weisen die roten Blutkörperchen auf, die man zweckmässig nach ihrem ausgebildeten Zustande in solche mit Dauerkernen und solche mit vergänglichen Kernen einteilen kann; die ersteren sind für die Nichtsäuger, die letzteren für die Säuger charakteristisch. Ausser der Verschiedenheit in dem Verhalten des Kernes ergeben sich aber auch Unterschiede in der Form; die roten Blutkörperchen mit Dauerkernen sind ovale, biconvexe Scheiben, nur bei den Cyclostomen finden sich napfförmige Gebilde; die roten Blutkörperchen mit vergänglichen Kernen sind convex-concav (napf- oder glockenförmig) und kreisrund, mit alleiniger Ausnahme der Tylopoden, wo sie eine ovale Grundform besitzen. Was die Struktur angeht, so

besteht zwischen beiden Formen weitgehende Uebereinstimmung; nach aussen sind sie von einer semipermeablen Membran, die Lecithin und Cholestearin enthält, abgeschlossen, während ihr Inneres im wesentlichen aus einer konzentrierten Hämoglobininlösung besteht; bei den Formen mit vergänglichem Kern ist irgend ein protoplasmatisches Innengerüst nicht vorhanden. Die Jugendformen beider Arten sind gleichfalls übereinstimmend, es sind kugelige Zellen mit rundem Kern; die Umwandlung in die definitive Form geht in der Weise vor sich, dass die Formen mit Dauerkernen allmählich oval werden, wobei auch der Kern diese Form annimmt, gleichzeitig aber in seinen chromatischen Teilen sich zusammenklumpt; gar nicht so selten geht der Process noch weiter, denn man begegnet gelegentlich Zellen, in denen kein Kern mehr nachweisbar ist. Bei den roten Blutkörperchen mit vergänglichen Kernen ist das die Regel, der Kern wird dabei in einzelne Stücke zerschnürt und verklumpt dabei zu mehr oder weniger homogenen Chromatinkugeln, die nach und nach ausgestossen werden; dabei geht die Zelle aus der runden Form in die Napfform über. Die Umbildung der roten Blutkörperchen stellt also vom morphologischen Gesichtspunkte aus betrachtet eine Degeneration dar; zur Erreichung höchster physiologischer Wirksamkeit geht Protoplasmastruktur und Kern verloren, das Plasma selbst bildet sich zu einer besonderen flüssigen Masse um, die nach aussen durch eine halbdurchlässige Membran abgeschlossen wird; bei den Blutkörperchen mit Dauerkernen ist der Endeffekt nicht so weitgehend, der Kern bleibt, wenn er auch zuletzt stark degenerativ verändert wird, in der Zelle, und auch das Plasma wird nicht völlig verflüssigt.

Weit vielgestaltiger als die roten sind die weissen Blutkörperchen. Aber wenn auch die einzelnen Formen in Grösse, Kern und Plasmastruktur verschieden sind, so besteht doch insofern eine Uebereinstimmung als die gleichen Typen im grossen und ganzen bei allen Wirbeltiergruppen wiederkehren. Für die Betrachtung der einzelnen Formen erscheint es dabei zweckmässig von den Säugetieren auszugehen. Man kann hier zunächst zweierlei Arten von Leucocyten unterscheiden, nämlich ungranulierte und granulierte Zellen, wobei unter Granulierung nicht eine gelegent-

lich so bezeichnete undeutliche Protoplasmastruktur verstanden wird, sondern das Vorhandensein distinkter d.h. scharf aus dem übrigen Plasmaleib hervortretender und auch am lebenden Objekt sichtbarer granulärer Einlagerungen.

Die ungranulierten und die granulierten Formen treten nun jede wieder in verschiedenen Typen auf und zwar lassen die ungranulierten zwei Arten unterscheiden, nämlich: 1, kleine Zellen mit grossem, meist rundem Kern und schmalem Plasmasaum—die Lymphocyten nach Ehrlich's Nomenklatur, und 2, grosse Zellen mit grossem, rundem oder auch eingebuchtetem Kern und breitem Plasmaleib—grosse Lymphocyten, grosse mononukleäre Leucocyten, Uebergangsformen der Ehrlichschen Nomenklatur. Die granulierten Zellen zerfallen in feingranulierte—auch als neutrophil, etc., oder spezial granuliert bezeichnet—in grobgranulierte—die oxyphilen, acidophilen oder eosinophilen der Autoren—und endlich die sog. Mastleucocyten, die basophil granulierten Leucocyten der Ehrlichschen Nomenklatur.

Betrachten wir zunächst die granulierten Formen, so ist bekannt dass Ehrlich das Verhalten der Granulationen zu bestimmten Farbstoffen als Einteilungsprinzip aufgestellt hat und in der Tat eignen sich die von Ehrlich angewandten besonderen Farbstoffe (Triacid, Eosin, Methylenblau) sehr gut zur Darstellung des differenten Färbungscharakters der Körnelung und damit auch zu einer Unterscheidung der einzelnen Formen zu einander. Allein Ehrlich ging weiter, er betrachtete die Affinität der Granula zu bestimmten Farbstoffen als den Ausdruck einer charakteristischen chemischen Reaktion; von dem Gedanken ausgehend, dass jeder Zelle nur eine bestimmte Funktion zukomme, die er wieder in der Ausarbeitung solcher Granulationen erblickte, stellte er den Lehrsatz von der spezifischen Natur der Granula und der sie tragenden Zellen auf. Er übertrug also das seiner Meinung nach rein chemische Einteilungsprinzip auch auf das morphologische und genetische Gebiet und leugnete auf Grund desselben einerseits jede Beziehung der tinktoriell verschieden granuliert erscheinenden Formen zueinander, während er andererseits die Formen mit tinktoriell gleichem Verhalten der Granula als zusammengehörig betrachtete. Ein Teil der Ehrlichschen

Schule hat diese Auffassung als Grunddogma angenommen, nämlich dass nur "Zellen der gleichen Art gleiche Granula hervorbringen" könnten, wobei aber unter gleichen Granula eben nur färberisch gleiche Granula verstanden werden.

Allein diese Lehre lässt sich nicht aufrecht erhalten. Angenommen die Art der Färbung sei wirklich der Ausdruck einer chemischen Reaktion, so ist damit doch über Art und Wesen der Reaktion gar nichts ausgesagt, und die eigentlich chemische Natur der Granula wurde uns infolgedessen auch in der Tat nicht um eine Spur klarer; solange wir aber nicht den chemischen oder physiologischen Charakter der Granulationen kennen, ist es unmöglich festzustellen, ob wirklich diese Protoplasmaeinlagerung eine ausschliessliche Eigentümlichkeit der fraglichen Zelle darstellt. Aber selbst wenn dem so wäre, so ist damit noch lange nicht gesagt, dass deswegen Zellen mit andren Granulationen nicht in genetischen Beziehungen zu ihnen stehen können oder dass vielleicht sogar auch die Ausarbeitung der Granulation nur der Ausdruck eines passageren Funktionszustandes ist. Wissen wir doch, dass in den gleichen Zellen die verschiedensten chemischen Umsetzungen möglich sind und zum Teil auch zum morphologisch-granulären Ausdruck kommen können, wie das z. B. bei der Leberzelle der Fall ist. Nun sind aber noch abgesehen von diesen allgemeinen, gegen die Gültigkeit und Verwertbarkeit der färberischen Besonderheiten sprechenden Erwägungen noch eine Reihe von Momente bekannt geworden, die in gleichem Sinne sprechen. So haben besonders Arnold und seine Schüler nachgewiesen, dass in der gleichen Leucocytenform verschieden färbbare Granula (z. B. basophile in eosinophilen) vorkommen können, eine in der Folge von Ehrlich selbst zugegebene Beobachtung, die gerade den Hauptsatz seiner Beweisführung umstösst; denn ist in der Tat der Färbungscharakter der Granulation artbestimmend für diese und die Zelle selbst, so können unmöglich in der gleichen Zelle zwei Granulaarten vorkommen, die sich mit genau denselben tinktoriellen Eigenschaften auf zwei sonst als spezifisch verschieden bezeichnete Zellformen verteilen. Dass man sich damit zu helfen suchte, und die färberisch abweichende Granulation als Jugendstadium bezeichnete,



kann daran nichts ändern; denn selbst wenn dem so wäre, so würde damit gerade bewiesen, dass der tinktorielle Charakter nichts feststehendes ist, sondern einem Wechsel unterliegt und somit als ein absolut sicheres Einteilungsprinzip für die einzelnen Zellkategorien erst recht nicht verwertbar ist. Besonders auch für die feingranulierten Leucocyten des Menschen, die man hier als neutrophile bezeichnet, wurde nachgewiesen, dass ihre Jugendformen nur Granula mit basophilem Färbungscharakter enthalten, die erst im Verlauf der weiteren Zellumbildung zu neutrophilen werden. Nun steht aber jetzt auch weiterhin fest, dass Granula mit gleichem Färbungscharakter in Zellen vorkommen, die morphologisch und genetisch nicht das geringste mit einander zu tun haben, und andererseits sind Zellen mit gleichen Granula deswegen weder morphologisch noch genetisch gleich. Als Beispiel für den ersten Fall sei daran erinnert, dass eosinophile Körnelung sowohl den Darmepithelien als auch den Zellen des Dotterentoderms eigen sein kann, ja um bei den Leucocyten zu bleiben, dass beim Kaninchen die feingranulierten, den neutrophilen oder spezialgranulierten Formen entsprechenden Elemente gleichfalls eosinophil sind, also den gleichen Färbungscharakter wie die grobgranulierten oder eosinophilen besitzen; dass man sie zur Unterscheidung von diesen als pseudoeosinophile bezeichnet hat, ist ein lediglich nomenklatorischer Notbehelf, der über die Tatsache selbst nicht hinwegtäuschen kann, dass eben beim Kaninchen fein- und grobgranulierte Leucocyten, die sonst auf Grund der verschiedenen Färbbarkeit morphologisch und genetisch scharf von einander getrennt werden, gleiche Farbenaffinität besitzen. Für den zweiten oben genannten Fall geben die Mastleucocyten ein sehr gutes Beispiel ab. Während man früher lediglich auf Grund des färberischen Einteilungsprinzips alle basophil granulierten Elemente als Mastleucocyten zusammenfasste, hat sich jetzt gezeigt, dass nicht nur die Mastzellen des Blutes und des Bindegewebes durchaus morphologisch von einander verschieden sind (Fig. 5, 6, u. 7), sondern dass auch die Mastzellen des Blutes, die sog. Mastleucocyten nach ihrem morphologischen Bilde zwei verschiedenen Typen angehören, die ich als Typus Mensch (Fig. 5) und Typus Meerschweinchen (Fig. 6) bezeichnet habe.

Aus all dem ergibt sich also, dass der färberische Charakter der Leucocytengranulation keinen sicheren oder gar allein verwertbaren Massstab zur Beurteilung der morphologischen und genetischen Beziehungen der verschiedenen Formen zu einander abgeben kann. Morphologische Gesichtspunkte sind es, die allein nur für die Beurteilung dieser Fragen in Betracht kommen können. Für die Granulationen selbst sind ausser der Art ihrer Färbbarkeit vor allem auch ihre Form, Grösse und Zahl bestimmend. Daneben ist es aber besonders der Kern, der ein wesentliches Kriterium und Charakteristikum des morphologischen Zellbildes abgibt.

Während man ursprünglich der Kernform keinerlei besondere Beachtung schenkte und die Anatomen gar geneigt waren anzunehmen, dass seine Form nicht konstant sei, sondern als Folge der amöboiden Bewegung mit dieser beliebig wechseln könne, habe ich zu zeigen vermocht, dass doch eine grosse Gesetzmässigkeit in der Kernform vorwaltet. Schon längst war bekannt, dass die jugendlichen Zellen speziell auch die der granulierten Leucocyten einen rundlichen, in sich geschlossenen d.h. aus einer einheitlichen Masse bestehenden Kern besitzen—mononukleäre Leucocyten—den ich als kompakt bezeichne (Fig. 1, *a*. u. 4, *a*). Diese Kernform erfährt nun eine Umbildung, deren Gründe noch unbekannt sind, die aber nicht in äussern Zufälligkeiten, wie die Art der Bewegung es ist, zu suchen ist, sondern in inneren Zellvorgängen, den Beziehungen der Kernsubstanz zum umgebenden Protoplasma. Der rundliche Kern wird zunächst nieren- dann hufeisenförmig und erscheint in seiner Gesamtheit wurstförmig peripher gelagert. Weiterhin wird die einheitliche Kernmasse gelappt und zwar derart, dass die einzelnen Lappen noch durch feine Fäden in Verbindung bleiben. Eine völlige Zerteilung des Kernes in isolierte Fragmente kommt im normalen Blute nicht vor, wohl aber im pathologisch veränderten oder an Leucocyten, die wie bei der Entzündung die Gefässbahn verlassen haben; solche fragmentierte Leucocyten sind als Degenerationsformen aufzufassen (Fig. 1, *g*, *h*, u. Fig. 4, *d*). Die Art der Lappung ist bei den einzelnen granulierten Formen durchaus und charakteristisch verschieden. Am einfachsten

verhalten sich hierin die grobgranulierten (eosinophilen) Elemente. Die Lappung besteht hier meistens in einer Zweiteilung, so dass der Kern hantel- oder zwerchsackförmig erscheint (Fig. 4, b, c, e); seltener sind drei Lappen; meist sind auch die beiden Lappen gleich oder nahezu gleich gross. Bei der Ratte und Maus hat der Kern dieser Zellen die Form eines gleichnässig dicken Ringes. Bei den feingranulierten Leucocyten ist die Lappung reichlicher, bis zu fünf Segmente kommen hier vor, und daraus erklärt sich auch die bei diesen Leucocyten zu beobachtende eigentümliche Verschiedenheit der Kernformen, die in einer nur durch die amöboide Bewegung bedingten Verlagerung der Lappen begründet ist. Während der gesammte, auch gelappte Kern seine periphere hufeisenförmige Lagerung zunächst beibehält (Fig. 1, b, d), werden durch die Bewegung die einzelnen Teile derart verlagert, dass Spiralen, S- oder Schleifenformen entstehen (Fig. 1, e, f). Wo in der Regel nur Zweilappung auftritt, wie bei den grobgranulierten Leucocyten, kommt es höchstens zur Bildung einer S-Form (Fig. 4, b). Ganz besonders verhalten sich die Mastleucocyten; während die vom Typus des Meerschweinchens in ihrer Kernform den feingranulierten ähneln (Fig. 6), besitzen die menschlichen im normalen Blute meist kompakte und wenig unregelmässige Kerne (Fig. 5, a, b); im pathologisch veränderten Blute (Leucämie) kann auch hier eine sehr starke Lappung eintreten, an der aber jede Gesetzmässigkeit vermisst wird; die Kerne werden hier vielmehr im einzeln ganz verschieden zerklüftet und fragmentiert (Fig. 5, e, f).

Wie die gleichzeitig mit der Lappung in der Kernstruktur nachweisbaren Veränderungen beweisen, bei denen die Tendenz zur Verklumpung und Homogenisierung bei gleichzeitigem Schwund der Kernkörperchen ganz unverkennbar ist, handelt es sich bei der Lappenbildung in letzter Linie auch um morphologisch degenerative Umwandlungen, mit denen der Kern seine Fähigkeit zur mitotischen Teilung verliert. Diese selbst ist ausschliesslich an den jugendlichen Zustand des kompakten, nicht gelappten Kerns gebunden. Dass gelappte Kerne infolge der Bewegung oder aus inneren Gründen wieder zu kompakten, rundkernigen Formen werden können, ist sicher auszuschliessen; die in der Literatur

niedergelegten entgegenstehenden Beobachtungen am lebenden Objekt beruhen auf Täuschung und sind dadurch zu erklären, dass bei der Bewegung die einzelnen Lappen gelegentlich in einen Haufen zusammenzuliegen kommen, der am unfixierten und ungefärbten Material dann als eine einheitliche Masse erscheinen kann.

Weiterhin zeigte sich aber auch, dass die Art der Lappung bei den einzelnen Formenganz charakteristisch ist. Die grobgranulierten Leucocyten weisen meistens nur zwei Lappen auf bei gleichzeitiger Konstanz der Kernlappung, während die feingranulierten viel reichlicher gelappt sind und infolgedessen auch vielgestaltiger und variabler in Art und Lagerung der Lappen. Die Mastleucocyten zeigen unter normalen Verhältnissen einfache kompakte Kerne. Es ist interessant und wichtig, dass diese Eigentümlichkeiten des Kernes nicht etwa auf den Menschen beschränkt sind sondern im Prinzip sich auch bei allen andren Säugetieren und auch bei den Amphibien nachweisen lassen.

Was nun die Morphologie der Granulationen angeht, so herrscht hier viel weniger Einheitlichkeit als bei den Kernformen. Anscheinend durch die ganze Reihe der Wirbeltiere sind grobgranulierte Leucocyten nachweisbar. Ihre Färbbarkeit mit Eosin hat dazu geführt, alle Elemente, die solche mit Eosin färbbare Einlagerungen besitzen als gleichwertig zu betrachten und demnach finden sich in der Literatur alle mit dieser Eigenschaft ausgestatteten Elemente so verzeichnet ohne Rücksicht auf die morphologischen Besonderheiten der Granula selbst. Ich habe schon oben darauf hingewiesen, dass beim Kaninchen sog. pseudoeosinophile Granula neben richtigen eosinophilen vorhanden sind, dass man aber wegen ihres zahlenmässigen Vorkommens und ihres ganzen biologischen Verhaltens die pseudoeosinophilen Leucocyten morphologisch und physiologisch den feingranulierten der übrigen Säuger gleichsetzen muss, vor allem auch deswegen weil diese selbst sonst vollständig fehlen würden. Eine ähnliche Vorsicht ist bei der Beurteilung der stäbchenförmigen oder kristalloiden Leucocyten geboten, die eosinophilen Färbungscharakter besitzen, aber in ihrem biologischen Verhalten eher den feingranulierten entsprechen dürften, wenn man überhaupt solche

Homologien aufstellen darf. Diese kristalloiden Granula finden sich in den Leucocyten mancher Fische (bei einzelnen Selachiern) und bei Vögeln, daneben kommen hier aber auch Leucocyten mit rundlichen eosinophilen Granulationen vor. Die bei den Säugern als eosinophil bezeichneten Granulationen sind meist rundlich, selten ellipsoid oder länglich und von verschiedener Grösse, bei den Pferden sind sie besonders voluminös. Am frischen Objekt, besonders deutlich an frischen Zupfpräparaten des Knochenmarks weisen sie eine gelbgrünliche, hämoglobinähnliche Färbung auf, die gerade auch beim Pferde besonders deutlich hervortritt—Semmers "rote Körnerkugeln." Bei Amphibien, ebenso wie bei Säugetieren, haben sie nicht selten eine mittlere helle, vakuolenartige Stelle, die bei seitlicher Betrachtung die Granulation napfförmig erscheinen lässt (Fig. 4, e).

Die Granula der feingranulierten Leucocyten, die beim Menschen neutrophilen Färbungscharakter aufweisen, variieren bei den Säugetieren in ihrem Verhalten zu den Farbstoffen sehr stark; beim Kaninchen sind sie pseudoeosinophil, beim Meerschweinchen amphophil, bei Maus und Ratte sind sie mit den üblichen Färbungsmethoden überhaupt nicht nachweisbar. Das letztere Verhalten erinnert an die entsprechenden Zellen der Amphibien, in denen sich bisher keine Granulationen nachweisen liessen. Hinsichtlich der Form und Grösse der Granula besteht grössere Uebereinstimmung, insofern sie bei allen Tieren, wo sie bisher beschrieben wurden, ziemlich klein, rund und von gleicher Grösse sind.

Die basophilen Granula der Mastleucocyten sind beim Menschen grob und sehr unregelmässig in Form und Grösse, auch ihre Zahl wechselt in den einzelnen Zellen ziemlich stark (Fig. 5, a-d). Beim Typus des Meerschweinchens sind die Granula sehr zahlreich, grösser wie die eosinophilen und von mehr ovaler gleichmässiger Form (Fig. 6, a, b). Interessant ist, dass sie bei den Amphibien, speziell bei den Anuren, sich mehr an den menschlichen Typus anlehnen (Fig. 5, g-k).

Ueber die Bedeutung der verschiedenen Granulationen ist noch wenig bekannt. Während wir die feinen Granula wohl als endogene Protoplasmabildungen ansehen dürfen, scheint bei den menschlichen Mastleucocyten die Bildung der Granula unter

Mitbeteiligung des Kerns vor sich zu gehen, was bei gleichzeitiger Dekomposition des Kernes auf irgend welche degenerative Umbildungsprozesse schliessen lässt. Doch dürfte das eben nur für die beim Menschen vorkommende Form Geltung haben.

Die eosinophilen Granula der Säugetiere sind als exogene Plasmaeinlagerungen zu bezeichnen und zwar als hämoglobinhaltige Teile, grösstenteils von Erythrocyten herrührend, die durch hämolytische Vorgänge zerstört, oder in toto phagocytiert wurden (Fig. 4, *f-k*).

Aus all dem ergibt sich also, dass die Granulationen der Leucocyten keineswegs gleichwertige Bildungen sind, für deren Beurteilung das Verhalten zu Farbstoffen einen irgendwie genügenden Anhaltspunkt böte. Nur das gesammte morphologische und biologische Bild der Zelle vermag Aufschlüsse über ihre Art und Bedeutung zu geben und daraus folgt jedenfalls soviel, dass die verschiedenen granulierten Leucocyten als einseitig differenzierte Zellen aufzufassen sind, deren Funktion und Lebensschicksal festgelegt ist. Ein Uebergang einer Zellform in die andre ist bisher nicht beobachtet worden und auch nicht wahrscheinlich. Diese spezielle Differenzierung hindert aber natürlich keineswegs anzunehmen, dass die Zellen aus indifferenten gleichen Formen fortdauernd ihre Entwicklung nehmen.

Viel weniger als die granulierten Leucocyten variieren die ungranulierten. Soviel bisher bekannt geworden ist, finden sich bei allen Wirbeltieren, wenn auch in wechselnder Zahl zweierlei Formen, die man als kleine und grosse bezeichnen kann, ohne dass aber etwa diese beiden Formen absolut unabhängig von einander wären; im Gegenteil lässt sich zeigen, dass bei allen Tieren Zwischenformen vorkommen, die gestatten eine kontinuierlich fortlaufende Reihe von den kleinen zu den grossen Formen aufzustellen. Die kleinen Formen, die nach Ehrlich jetzt fast allgemein als Lymphocyten bezeichnet werden, sind durch einen verhältnissmässig grossen, meist runden Kern und schmalen Plasmaum charakterisiert, das Plasma besitzt mehr oder weniger ausgesprochen basophilen Färbungscharakter (Fig. 8, *a-d* u. *l*). Die grossen Formen haben einen grossen, rundlichen, bohnenförmigen oder sonst eingekerbten Kern, der aber niemals—unter

normalen Verhältnissen—Lappenbildung zeigt, wie sie für die granulierten Formen charakteristisch ist; das Plasma ist breit und enthält bei manchen Tieren, so besonders beim Meerschweinchen, eigentümliche grobe Einlagerungen—sog. Kurloffsche Körperchen—über deren Natur nichts bestimmtes bekannt ist (Fig. 8, *e-i* u. *m*). Sowohl bei den kleinen wie bei den grossen Formen finden sich gelegentlich, oft sehr selten und unregelmässig, vereinzelte Granulationen, die nur mit bestimmten Farbstoffen darstellbar sind und die als azurophil beschrieben werden; über ihre Bedeutung ist nichts genaues bekannt. Die grossen Formen gehen unter den verschiedenen, oben aufgeführten Namen.

Was nun die Beziehungen der verschiedenen Leucocytenformen zu einander und ihre Herkunft angeht, so haben die älteren Anatomen angenommen, dass sie alle eines Ursprungs sind und aus den Lymphdrüsen entstehen. Erst Ehrlich hat auf Grund seiner färbungsdiagnostischen Einteilung auch örtlich und genetisch eine Trennung der einzelnen Formen vorgenommen, und zwar verlegte er den Ort der Entstehung der kleinen Lymphocyten in die Lymphdrüsen, die der granulierten in das Knochenmark und für die grossen ungranulierten Elemente liess er es ungewiss, ob sie aus Knochenmark oder Milz stammen. Aber auch genetisch sollten die verschiedenen Formen nichts miteinander zu tun haben, sondern wieder aus speziellen differenzierten Elementen auf dem Wege der Mitose hervorgehen. Diese letztere Auffassung gewann in der Folge namentlich dadurch an Boden, dass es gelang, in granulierten Leucocyten des Knochenmarks Mitosen nachzuweisen und zwar in den grob- und feingranulierten der Säugetiere; aus den im Knochenmark stets vorhandenen granulierten Mutterzellen, die durch einen einheitlichen kompakten Kern charakterisiert sind—mononukleäre Leucocyten, sog. Myelocyten—(Fig. 1, *a*, u. Fig. 4, *a*), sollten so durch Teilung die eigentlichen Leucocyten immer neu gebildet werden. Auch hier hat man auf Grund einzelner Beobachtungen wieder verallgemeinert: da beim Meerschweinchen in den Mastleucocyten des Knochenmarks Mitosen festgestellt wurden, schloss man ohne weiteres, dass alle Mastleucocyten auf gleichem Wege im Knochenmark entstünden und doch ist heute sicher,

dass beim Menschen die Mastleucocyten einen ganz anderen Typus zeigen, und ebenso ist über ihr vermehrtes Vorkommen im Knochenmark und gar über mitotische Teilung nichts bekannt geworden. Diese ursprüngliche Lehre, dass granulierten Leucocyten auch postembryonal nur auf mitotischem Wege aus ebenso granulierten entstehen, ist bald erschüttert worden und zwar durch den Nachweis ungranulierter Elemente von lymphocytärem Charakter im Knochenmark selbst; diese Zellen, die man als Markzellen, Promyelocyten, etc., bezeichnet hat, sollen in ihrem basophilen Plasma Granula von zunächst basophilem Färbungscharakter ausarbeiten und so zu Myelocyten werden. Auch hierbei wurde wieder insofern verallgemeinert, als die für die feingranulierten Elemente festgestellten Tatsachen ohne weiteres auf die grobgranulierten und die Mastleucocyten übertragen wurden.

Die kleinen Lymphocyten sollten nach diesen Lehren in den Lymphdrüsen und zwar zunächst ausschliesslich dort aus den grossen Keimzentrumszellen der Sekundärknötchen entstehen. Eine weitere Entwicklung aber wurde gelehrt, die Lymphocyten sollen stets nur Lymphocyten bleiben; nur in pathologischen Fällen soll eine degenerative Weiterbildung in der Form stark buchtkerniger Zellen möglich sein, die man als Riedersche Zellen bezeichnet hat. Die Keimzentrumszellen selbst sollen normaler Weise überhaupt nicht, höchstens bei Kindern in die Zirculation gelangen. Noch weniger wusste man mit den grossen ungranulierten Zellen anzufangen; zwar nahm Ehrlich anfangs selbst an, dass sie zu granulierten werden können, aber seine Schule hat diese Ansicht bald aufgegeben und die Zellen als selbstständige Gebilde betrachtet, die man weil sie besonders aus der Milz stammen sollen, späterhin auch mit dem Namen der Splenocyten belegte.

Die Auffindung ungranulierter lymphocytenähnlicher Elemente im Knochenmark führte zur Aufwerfung der Frage, ob dieses Element identisch ist mit den grossen Lymphocyten der Keimzentren. Während die einen diese Identität auf Grund der allgemeinen morphologischen Uebereinstimmung der beiden Formen und gestützt auf die Tatsache, dass diese Zellen neben granu-



lierten Leucocyten auch typische Lymphocyten im Knochenmark aus sich hervorgehen lassen, behaupten, bestritten andre jegliche Identität. Von vielen wird dabei zwar die morphologische Unmöglichkeit der Unterscheidung zugegeben, aber doch die Verschiedenheit der Art aus der angeblichen Divergenz der Differenzierungsprodukte abgeleitet. Andre wieder, so besonders Nägeli und Schridde, suchen nach morphologischen Unterschieden zwischen den beiden Ausgangsformen. Während Nägeli ursprünglich die Zahl der Nukleolen verwerten wollte, kam er später selbst wieder davon ab, nachdem er einsehen musste, dass bei der notorischen Inkonstanz dieser Gebilde und ihrer Variabilität bei beiden Formen diesen Kriterien keinerlei Bedeutung zukommen kann. Viel Wert wurde dann besonders von Schridde auf das Vorhandensein Altmannscher Granula in den lymphocytären Elementen der Lymphdrüsen gelegt, die in den Promyelocyten des Knochenmarks fehlen sollten. Ganz abgesehen davon, dass aber solche Granula schon früher von Ceconi und neuerdings von Walgren und E. Meyer auch in den Promyelocyten des Knochenmarks nachgewiesen wurden, handelt es sich bei dieser Bildung um keinerlei artbestimmendes Merkmal. Benda hält diese Körnelung für Mitochondrien, wie sie sich in allen wachsenden Zellen nachweisen lassen. Es scheint, dass neuerdings auch Schridde selbst von seiner Wertschätzung dieser granulären Bildungen abgekommen ist, denn er bezeichnet jetzt den Kern und nicht mehr die Granula als "Wappen" der Zelle.

Somit steht heute fest, dass die ungranulierten Zellen des Knochenmarks, die weiterhin sich in die granulierten Formen differenzieren, und die grösseren Lymphocyten der Lymphdrüsen morphologisch durchaus identische Elemente sind. Aber diese Identität kann aber noch auf andrem Wege erwiesen werden. Wie wir wissen, dass im Knochenmark aus den gleichen Formen auch typische Lymphocyten gebildet werden können, sowohl unter normalen wie besonders auch unter pathologischen Verhältnissen, so lässt sich auch andererseits zeigen, dass die im lymphoiden Gewebe gebildeten Lymphocyten weiter differenzierungsfähig sind und zwar auch in der Richtung der granulierten Leucocyten.

Man weiss schon längst, dass die kleinen Lymphocyten der Ehrlichschen Nomenklatur aus den grossen Formen, den Keimzentrumszellen, durch mitotische Teilung hervorgehen, andererseits haben aber die neueren Untersuchungen ergeben, dass diese kleinen Formen selbst wieder zu grossen teilungsfähigen Elementen heranwachsen können. Das lässt sich nicht nur bei Entzündungen zeigen, wie das Maximow, Helly, Blumenthal, Schott und ich getan haben, sondern das wird auch dadurch erwiesen, dass in der normalen Lymphe stets Zellen in Menge angetroffen werden, die sowohl in Zellgrösse wie Kern und Plasmacharakter als kontinuierliche Uebergangsformen zwischen den grossen und den kleinen zu gelten haben (Fig. 8, *a-i*), und damit kommen wir auch zu einer Erklärung für die grossen lymphocytären Elemente des strömenden Blutes, deren fragliche Herkunft Ehrlich entweder in das Knochenmark oder in die Milz verlegt hat. Untersucht man nämlich die Lymphe des Ductus thoracicus, so findet man nicht nur diese grossen Formen in Menge, sondern auch reichliche Mitosen in ihnen (Fig. 8, *h*) und die eben erwähnten Uebergänge zu den kleineren Formen (Fig. 8, *a-i*). Daraus folgt, dass die sog. grossen mononukleären Leucocyten Ehrlichs jedenfalls auch aus der Lymphe stammen und wie ihre Fähigkeit zur mitotischen Vermehrung erweist, identisch sind mit den auch morphologisch mit ihnen übereinstimmenden Keimzentrumszellen. Die kleinen und die grossen ungranulierten Elemente gehören also zusammen, es sind Lymphocyten verschiedener Grösse; die kleinen Formen sind lediglich als der Ausdruck einer besonders lebhaften artproduktiven Zelltätigkeit zu betrachten, wie wir denn auch sonst sehen, dass Zellen bei rasch fortgesetzten Teilungsvorgängen an Grösse abnehmen. Nun stammen aber die grossen Formen nicht ausschliesslich aus lymphoidem Gewebe; sie finden sich ebenso in Knochenmark und Milz, vor allem aber sind mit ihnen auch die Zellen identisch, die man im Transudat seröser Höhlen antrifft (Fig. 9), wo sie besonders unter entzündlichen Einflüssen in der Form der Makrophagen ein wesentliches Zellelement darstellen. Man hat sie früher hier vielfach für emigrierte und stark vergrösserte Blutlymphocyten gehalten oder auch für abgestossene und degenerierende Deckzellen der Serosa.

Nach den Untersuchungen Schotts steht aber fest, dass sie nicht sämtlich aus dem Blute stammen, sondern zum grösseren Teil losgelöste Deckzellen besonders des Netzes sind, die aber nicht degenerieren, sondern im Gegenteil lebenskräftige, mitotischer Teilung fähige Elemente sind (Fig. 9, c), mit der Aufgabe, die bei Entzündungen in die serösen Höhlen gelangenden granulierten Leucocyten oder Fremdkörper in sich aufzunehmen. Sie entsprechen demnach den grossen phagocytierenden Zellen der lymphoiden Organe. Aber auch im Blute selbst können diese Elemente ihren phagocyitären Charakter entfalten, so ist vor nicht langer Zeit ein Krankheitsfall durch Rowley bekannt geworden, bei dem eben die grossen mononukleären Leucocyten andre Zellen in grossem Massstab in sich aufgenommen hatten.

Somit kommen wir also zu dem Ergebniss, dass die ungranulierten Leucocyten, die kleinen und die grossen Formen zusammengehören und die gleichen morphologischen und physiologischen Merkmale besitzen wie die entsprechenden Zellen des lymphoiden Gewebes, dass sie aber auch den undifferenzierten Elementen des Knochenmarks gleichwertig sind. Von diesen wissen wir, dass sie sich in der Richtung der granulierten Leucocyten differenzieren können, das gleiche trifft nun aber auch für die beiden Formen der lymphocytären Elemente zu und zwar ist dieser Nachweis für jede Form der granulierten Leucocyten zu führen.

Ein typisches Beispiel für die Fähigkeit lymphocytärer Elemente, die ihren Ursprung aus einwandfrei lymphoidem Gewebe nehmen, sich in feingranulierte Leucocyten umzuwandeln, liefern die sog. Speichelkörperchen. Wie ich zeigen konnte, sind diese Zellen, deren Granulagehalt schon den ältesten Beobachtern nicht entgangen war, feingranulierte (beim Menschen neutrophile) Leucocyten. Nicht nur stimmen ihre Granula mit den diesen Zellen eigentümlichen in Zahl, Form, Grösse und Färbungscharakter durchaus überein, sondern auch die Kerne weisen die charakteristische Lappung auf, wenn auch bei vielen die durch die Speichelflüssigkeit bedingte Quellung das Bild der einzelnen Lappen verwischt (Fig. 3). Andererseits finden sich aber auch unter ihnen Elemente mit kompaktem Kern, die in ihrem ganzen morphologischen Verhalten den Myelocyten des Knochenmarks entsprechen.

Demgemäss hat Ehrlich selbst die Speichelkörperchen früher als neutrophile Myelocyten bezeichnet. Die Gegner dieser Auffassung, die diese Differenzierungsmöglichkeit der Lymphocyten leugnen, sehen die kompaktkernigen Formen als Degenerationsprodukte der gelapptkernigen an, die sie als feingranulierte Leucocyten aus dem Blute ableiten. Aber abgesehen davon, dass es überhaupt keine Degeneration gibt, die aus gelapptkernigen Formen kompaktkernige vom morphologischen Typus der entsprechenden Speichelkörperchen hervorgehen lässt, ist dieser Einwand schon deswegen bestimmt unrichtig, weil er von der irrthümlichen, dem Anatomen unverständlichen Auffassung ausgeht, dass die Speichelkörperchen emigrierte Blutleucocyten seien und nicht aus dem lymphoiden Gewebe der Tonsillen stammen. Noch deutlicher lässt sich aber die Entwicklungsmöglichkeit der Lymphocyten im Amphibienblut nachweisen. Die den feingranulierten Leucocyten entsprechenden Elemente entbehren hier jeder Granulation, dagegen zeigen sie die charakteristische Lappung und Umbildung des Kernes (Fig. 2, e, f). Es lässt sich nun im Blute junger Amphibien zeigen, dass diese gelapptkernigen Leucocyten in kontinuierlicher Reihe mit den lymphocytären Elementen zusammenhängen, die ihrerseits durchaus den im Knochenmark nachweisbaren Zellen entsprechen (Fig. 2, a-f).

Ebenso wie die feingranulierten Leucocyten nehmen aber auch die grobgranulierten (eosinophilen) ihre Entstehung aus Lymphocyten. Ich habe schon früher gezeigt, dass in den Blutlymphdrüsen, besonders des Schafes und der Ratte, alle Uebergänge zwischen Lymphocyten und typischen zwerchsackförmigen eosinophilen Leucocyten nachweisbar sind. Ich will hier auf den gleichzeitig geführten Nachweis, dass die eosinophilen Granula selbst nichts andres sind als die Zerfallsprodukte in besonderer Weise hämolysierter Erythrocyten nicht weiter eingehen, da es sich hier nur um den Nachweis der Fähigkeit der Differenzierung der Lymphocyten überhaupt handelt. In neuerer Zeit gelang es mir auch zu zeigen, dass durch Einwirkung entsprechender Reize die lymphoiden Zellhaufen der *Taches laiteuses* im Netz von Kaninchen sich in Unmassen typischer eosinophiler Leuco-

cyten umzuwandeln vermögen. Gerade hierbei ist mit absolut Bestimmtheit der von den Leugnern dieser Differenzierung erhobene Einwand der Emigration aus der Blutbahn zu widerlegen, da einerseits weder die eosinophilen Leucocyten des Blutes vermehrt erscheinen noch in der Auswanderung sich befinden, andererseits aber die eosinophilen Leucocyten sich strikte an die Taches laiteuses halten und in der vorherrschenden kompakt-kernigen Form alle charakteristischen Merkmale des Kernes der kleinen Lymphocyten erkennen lassen (Fig. 4, *f-k*). Inzwischen sind diese meine Angaben auch von Dominici und Pappenheim bestätigt worden.

Was für die fein- und grobgranulierten Leucocyten gilt, hat aber auch für die Mastleucocyten seine Berechtigung. Speziell für die des Menschen konnte ich den Nachweis erbringen, dass sie durch kontinuierliche Uebergänge mit Lymphocyten in Zusammenhang stehen; die Zwischenformen sind durch Zellen mit kompaktem Kern und sehr spärlichen Granula ausgezeichnet, ebenso deutlich treten diese Beziehungen im Amphibienblute hervor, wo bei jungen Tieren auch in der Zirculation die ganze Entwicklung der lymphocytären Elemente zu Mastleucocyten nachweisbar ist (Fig. 5, *g-k*).

Die Möglichkeit der Umwandlung der Leucocyten zu granulierten Leucocyten und zwar nicht nur der undifferenzierten Form des Knochenmarks, sondern auch der im typischen lymphoiden Gewebe gebildeten Elemente ist nicht ernstlich mehr bestreitbar. Daran ändert auch die Tatsache nichts, dass wir heute noch nicht in der Lage sind, die Gründe für die Differenzierung zu erkennen und die örtlichen Ursachen, die sie auslöst. Auch darüber ob alle Lymphocyten sich in gleicher Richtung entwickeln, vermögen wir vorerst nichts zu sagen; aber die Fähigkeit der Weiterentwicklung in dieser Richtung unter Einwirkung besonderer Reize ist zweifellos diesen Zellen gegeben. Diese Fähigkeit ist nun aber noch eine viel weitergehende. Man hat früher gerade auf anatomischer Seite und besonders unter dem Einfluss der Bizzozeroschen Untersuchungen angenommen, dass die Erythrocyten seit ihrer ersten embryonalen Entwicklung einen scharf von den Leucocyten getrennten Stamm von Blutzellen darstellen, die

sich fortdauernd aus sich heraus lediglich auf dem Wege der Mitose hämoglobinhaltiger Mutterformen vermehren. Diese Auffassung fand vor allem ihre Stütze in älteren embryologischen Beobachtungen, wonach die Erythrocytenbildung zeitlich der der Leucocyten vorausgehe, und die Feststellung von Mitosen hämoglobinhaltiger Zellen im Knochenmark durch das ganze Leben hindurch leistete dieser Annahme Vorschub. Allein gerade im Knochenmark lässt sich zeigen, dass jenes undifferenzierte Element durch kontinuierliche Uebergänge nicht nur mit den granulierten Leucocyten, sondern auch mit den Erythrocyten oder besser zunächst mit den Erythroblasten in Zusammenhang steht. Noch deutlicher gelingt dieser Nachweis bei den Amphibien; hier finden sich im strömenden Blute junger Tiere alle nur wünschenswerte Uebergangsformen zwischen den Lymphocyten einerseits und den ausgebildeten roten Blutkörperchen andererseits (Fig. 10, *a-g*), und zwar in grossen Mengen. Was aber besonders noch zu Gunsten dieser Entwicklung spricht, ist die gleichzeitig zu konstatierende Tatsache, dass die mitotische Vermehrung der Erythrocyten nicht an ein bestimmtes Stadium des Hämoglobingehaltes gebunden ist, was notwendiger Weise der Fall sein müsste, wenn dieser Stoff als ein nur durch Teilung übertragbares und den Artcharakter der Zelle auch genetisch bestimmendes Zellorgan anzusehen wäre, sondern dass die Mitosen in jedem Moment dieser Entwicklung und somit durchaus unabhängig vom Hämoglobingehalt selbst und seiner Stärke nachweisbar sind (Fig. 10, *h-k*). Alles in allem also: Die Lymphocyten besitzen auch die Fähigkeit der Differenzierung in der Richtung der roten Blutkörperchen, allerdings ist auch hierbei das gleiche zu berücksichtigen, was schon oben über die Differenzierung der granulierten Leucocyten gesagt wurde, dass nämlich die auslösenden Ursachen der Entwicklung nicht an allen Orten und allen Zeiten vorhanden zu sein brauchen.

Die hier vertretene Ansicht einer einheitlichen Genese aller Blutzellen aus einem indifferenten, in der Form der verschiedenen Lymphocyten stets in der Circulation vorhandenen Element findet auch noch ihre Stütze durch das Experiment und die ontogenetische Entwicklung. Es ist schon längst bekannt, dass Knochen-

marksgewebe im späteren Leben und bei ganz gesunden Individuen sich an Oertlichkeiten etablieren kann, wo es ursprünglich nicht vorhanden ist, und dass dieses Gewebe, alle charakteristischen Elemente des Knochenmarks umfassend, sowohl rote wie weisse Blutkörperchen produzieren kann. Nimmt man an, dass jede Form der Blutzellen ihre eigene durchaus schon differenzierte Mutterzelle nur im Knochenmark habe und dass diese Zellen unter normalen Verhältnissen niemals in die Zirculation gelangen können, so ist das postembryonale Auftreten eines derartigen Gewebes vom Charakter des Knochenmarks an fremden Oertlichkeiten völlig unverständlich. Ganz anders dagegen bei der Voraussetzung, dass dauernd in der Zirculation und zwar in der Form der lymphocytären Elemente Zellen vorhanden sind, die die Differenzierungsmöglichkeit in der behaupteten Richtung besitzen und unter geeigneten, uns allerdings unbekannten Umständen auch diese Fähigkeit erweisen. So erklärt sich ungezwungen das Auftreten von typischem Knochenmarksgewebe in den bei zunehmendem Alter verknöchernden Kehlkopfknorpeln. Die Richtigkeit dieser Annahme hat Maximow auf dem Wege des Experiments erhärtet; er konnte nämlich zeigen, dass in der Tat in dem Knochenmarksgewebe, das nach Unterbindung der Nierenarterie in der hierbei auftretenden Knochenschale zur Ausbildung gelangt, die typischen Zellformen ihre Entstehung aus den undifferenzierten lymphocytären Elementen nehmen.

Für die Einheitlichkeit des Ursprungs aller Blutelemente aus einer undifferenzierten Form sprechen auch die neueren Ergebnisse der Entwicklungsgeschichte. Zwar haben die Anhänger der polyphyletischen Ursprungstheorie der Leucocyten auch nach Beobachtungen gesucht, die hier zu ihren Gunsten sprechen sollen; in diesem Sinne wurde besonders das angeblich frühere Auftreten der Erythrocyten verwertet. Auch wurde behauptet, dass die myeloiden Zellen, also die granulierten Elemente und deren ungranulierte Vorstufen früher entstünden als die Lymphocyten. Allein es wurde schon gezeigt, dass morphologisch ein Unterschied zwischen den Lymphocyten und jenen ungranulierten Vorstufen der granulierten Leucocyten gar nicht nachweisbar ist, sodass die Behauptung die erst auftretenden Leucocyten seien

“Myeloblasten” eine durch keine Tatsachen belegbare, absolut willkürliche Annahme darstellt. Im Gegensatz hierzu haben Maximow und Dantschakoff, ebenso wie früher schon Bryce gezeigt, dass von allem Anfang an bei der Differenzierung der Blutelemente ein Teil der Zellen kein Hämoglobin ausarbeitet, also farblos bleibt und so den morphologischen Charakter lymphocytärer Elemente zeigt; diese Formen lassen sich auch dauernd in der Zirculation nachweisen, besonders aber in den speziell blutbildenden Organen. Also gerade Zellen von lymphocytärem Charakter sind neben hämoglobinhaltigen von Anfang an vorhanden und auch als Mutterzellen der hämoglobinhaltigen zu betrachten. Die ersten granulierten Leucocyten treten im Bindegewebe auf und nehmen ihren Ausgang aus ungranulierten lymphocytären Formen. Speziell auch in der Leber entstehen die ersten Leucocyten aus Zellen die zugleich die Mutterzellen der roten sind, diese Auffassung Maximows hat in neuester Zeit Mollier bestätigt. Somit spricht die Entwicklungsgeschichte durchaus in gleichem Sinn wie die Ergebnisse der Untersuchung der postembryonalen Verhältnisse.

Wir kommen also zu dem Ergebniss, dass rote und weisse Blutkörperchen in engen genetischen Beziehungen zu einander stehen und zwar derart, dass eine gemeinsame indifferente Mutterzelle vorhanden ist, die die Fähigkeit besitzt, sich nach den verschiedenen Richtungen hin zu differenzieren. Diese Mutterzelle ist aber nicht nur embryonal nachweisbar, sondern sie erhält sich das ganze Leben hindurch und trägt dadurch zur dauernden Regeneration der fortwährend ausscheidenden Blutelemente bei; dass die einzelnen differenzierten Formen sich daneben noch mitotisch weitervermehren, kann nicht als Einwand gegen die Entwicklungsmöglichkeit geltend gemacht werden. Die Ausgangsform zeigt den morphologischen Typus der ungranulierten Leucocyten, d.h. der lymphocytären Elemente, die im ausgebildeten Organismus als kleine und grosse, aber doch stets durch kontinuierliche Uebergänge verbundene Formen vorkommen. Wie schon oben gesagt wurde, hängt diese Variabilität im Typus mit der besonders lebhaften Produktionstätigkeit dieser Elemente zusammen. Aber sowohl kleine wie grosse Lymphocyten be-



sitzen die Differenzierungsmöglichkeit, wenn sie auch abhängig ist von bestimmten, uns grösstenteils unbekannten und im umgebenden Milieu zu suchenden Ursachen. Dieselbe Zelle vermag postembryonal im Knochenmark Erythrocyten und Leucocyten zu produzieren, dagegen nicht oder nur in geringem Umfang in der Milz, Lymphdrüsen oder in sonstigem lymphoiden Gewebe. Sie kann aber diese Fähigkeit auch hier wieder erwerben, wenn die Zellen unter die Wirkung spezieller Reize gelangen. Bei bestimmten Krankheiten tritt so die "myeloide" Umwandlung der Milz und der lymphoiden Organe auf, im verknöchernden Knorpel entsteht so typisches Knochenmarkgewebe, die lymphoiden Zellenhaufen des Netzes verwandeln sich so in Haufen eosinophiler Leucocyten.

Die alte ursprüngliche Ansicht des einheitlichen genetischen Charakters aller Blutzellen erweist sich damit als richtig. Aber das ist jedenfalls von der neueren Lehre festzuhalten, dass die einmal differenzierten Zellformen dauernd als solche bestehen bleiben oder in dieser Form zu Grunde gehen. Rote Blutkörperchen oder gar etwa ihre Kerne können nie zu Leucocyten werden, aber auch die verschiedenen granulierten Formen weisen keinerlei Uebergänge untereinander auf. Entwicklungsfähig sind allein nur die lymphocytären Elemente. Dass gerade diese in gewissem Sinne das jugendliche regenerationsfähige Element der Blutzellen darstellen, geht auch aus den vergleichend anatomischen Untersuchungen hervor. Die neueren Erfahrungen schon an den Blutzellen der Wirbellosen, die wir Kollmann verdanken, lehren, dass trotz der grossen Mannigfaltigkeit der granulierten Formen, die grösstenteils in denen der Wirbeltiere kein deutliches Analogon haben, alle aus granulierten, lymphocytären Elementen ihren Ursprung nehmen. In der Reihe der Wirbeltiere selbst lässt sich feststellen, dass bei Fischen, Amphibien und Vögeln die Lymphocyten fast überall gegenüber den granulierten oder gelapptkernigen Formen der Leucocyten das vorherrschende Zellelement darstellen, ja bei manchen Fischarten finden sie sich fast ausschliesslich. Sehr wesentlich in diesem Sinne ist auch die Feststellung, dass bei jugendlichen Individuen der gleichen Art die Lymphocyten an Zahl überwiegen oder wenigstens in einem

viel grössern Prozentsatz vorkommen als im zunehmenden Alter. Das trifft nicht nur für die Amphibien zu, wie Freidsohn zeigte, sondern auch für den Menschen. Mag auch im einzelnen noch manches der näheren Untersuchung und Aufklärung bedürfen, so ist doch aus den Feststellungen der letzten Jahre die Tatsache des monophyletischen Ursprungs aller Blutzellen als gesichert zu betrachten und die Erkenntniss, dass die lymphocytären Formen das undifferenzierte, regenerationsfähige Element repräsentieren.

## FIGURENERKLÄRUNG

1. Feingranulierte (neutrophile) Leucocyten: **a**, kompaktkernige Form des Knochenmarks, sog. mononukleärer Leucocyt oder Myelocyt; **b-f**: Umbildung des Kernes, und zwar: **b**, ungelappte Hufeisenform; **d**, gelappte Hufeisenform; **c**, amöboide Bewegung; **e**, S-Form; **m**, Schleifenform; **g**, und **h**, fragmentierte degenerierende Zellen aus entzündlichen Exsudaten.

2. Gelapptkernige Leucocyten der Amphibien in ihrer Entwicklung aus lymphocytären Elementen.

3. Speicheldrüsenkörperchen des Menschen: **a** und **b**, mononukleäre Formen; **c** und **d**, Lappenbildung; **e**, gequollene Kernlappen.

4. Grobgranulierte (eosinophile) Leucocyten: **a**, kompaktkernige Form des Knochenmarks, sog. mononukleärer Leucocyt oder Myelocyt; **b**, und **c**, typische Zwerchsackformen; **d**, Degenerationsform; **e**, aus dem Blute eines Bufo; **f, g**, zu eosinophilen Leucocyten umgewandelte Lymphocyten aus den Taches laiteuses des Kaninchennetzes, und zwar: **f-i**, typische Lymphocytenkerne; **i**, in toto phagocytierter Erythrocyt; **k**, Schatten eines ausgelauchten Erythrocyten mit umgebenden eosinophilen Granulationen.

5. Mastleucocyten—basophil granulierte Leucocyten. **a-f**, menschliche Formen, und zwar: **a** und **b**, aus normalem Blute; **c-f**, aus pathologisch verändertem Blute **g, k**, aus dem Blute von Amphibien; **g**, lymphocytäre Form mit beginnender Granulabildung.

6. Mastleucocyten des Blutes vom Meerschweinchen.

7. Mastzellen des Bindegewebes: **a**, aus dem Peritonealtranssudat der Ratte; **b**, aus dem Netz des Meerschweinchens.

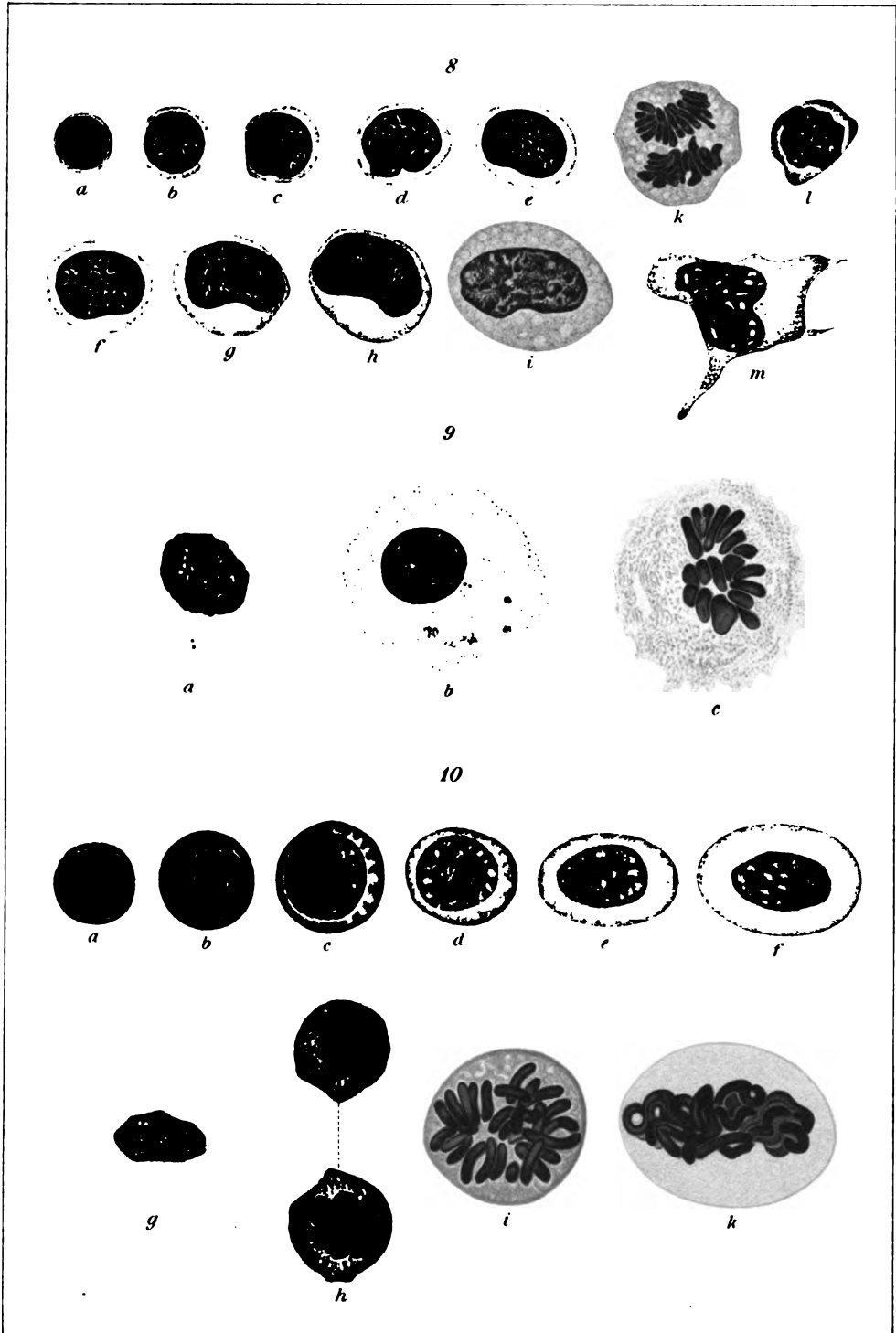
8. Kleine und grosse lymphocytäre Formen: **a-i**, kontinuierliche Reihe von Uebergängen zwischen den kleinen Lymphocyten und den grossen mononukleären Leucocyten aus der Lymphe des Kaninchens; **k**, Mitose einer grossen Form ebendaher; **l**, Lymphocyt aus dem menschlichen Blute; **m**, grosse Form, grosser mononukleärer Leucocyt aus dem menschlichen Blute in amöboider Bewegung.

9. Grosse lymphocytäre Formen aus dem Peritonealtranssudat des Kaninchens; **a** und **c**, normales Transsudat; **c**, Mitose; **b**, aus entzündlichem Exsudat.

10. Entwicklung roter Blutkörperchen aus lymphocytären Elementen: **a**, lymphocytäre Ausgangsform. Alle Zellen aus dem strömenden Blute junger Bufonen; **h-k**, Mitosen in Entwicklungsformen. Die Fähigkeit zur mitotischen Teilung ist unabhängig vom Grade des Hämoglobingehaltes.









# A FURTHER STUDY OF THE HUMAN UMBILICAL VESICLE

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## FOUR FIGURES

The material for this study consists of a well-preserved umbilical vesicle of a 13 mm. human embryo. The specimen was sent to me in a 5 per cent solution of formalin immediately after hysterectomy, by Dr. J. L. Crenshaw of Charlottesville, Va. It was at once transferred to 95 per cent alcohol, and subsequently embedded in paraffin and sectioned at 10 microns. The sections were stained in saffranin. The perfectly normal condition of the embryo itself and of the chorionic villi would seem to preclude all possibility of post-mortem degeneration, pathologic variation or fixation artifacts.

Primarily the object was simply a morphologic study of the entodermal tubules of this stage, supposed to be just past the phase of maximum development. Hence differential stains were not employed. This is to be regretted since numerous blood islands, showing with especial clearness the earliest stage in hematopoiesis, were subsequently discovered. The main criteria for a differentiation of these cells must consequently be morphologic, *e.g.*, presence of pseudopodia (indicating amoeboid motility), shape of nucleus, comparative size of nucleus and cell, granular character of cytoplasm, etc. Staining capacity of the protoplasm, however, yields consistent confirmatory information. Thus a certain type of cell always has a deeply staining homogeneous (hæmoglobin-containing) cytoplasm. The points of special interest in this study concern the tubules ("glands"—Spee; "crypts"—Selenka) and the blood islands.



An exceptional importance attaches to the human umbilical vesicle of this stage of development and for two reasons: (1) The tubules have just entered upon a functional decline. They appear to be at the height of their development and activity in vesicles of embryos of about 9 mm. (Spee, Meyer, Jordan, Branca.) (2) Schridde proposes to divide hæmatopoietic phenomena in the human embryo into two sharply defined periods. The earliest period ends at about the 10 to 12.5 mm. stage of growth. Meanwhile the blood cells (all of a single type, "primary erythroblasts" with hæmoglobin) have origin in blood channels. "Bluträume," of the umbilical vesicle and connecting-stalk. Moreover the original cells arise only from "vessel-wall cells" and proliferation is strictly intravascular. In embryos of 13 mm. the liver has assumed the hæmatopoietic function. Three different types of cells are said to arise simultaneously from the outer layers (*i.e.*, extravascular) of the hepatic capillaries: Myeloblasts, secondary erythroblasts, and giant cells. The secondary erythroblasts are told from the primary by their smaller size. Both from the standpoint of material and results, Schridde's position is unique. On the basis of very extensive observations on material from various higher mammals, prepared with almost faultless technique, Maximow rejects Schridde's hypothesis. Furthermore, as regards the source of the blood cells during the stages of hepatic hematopoiesis, these two investigators disagree. Maximow derives the blood mother-cells ("hæmatogonia," "lymphocytes," "primary wandering cells" of Saxer) from the mesenchyme. Schridde absolutely denies the presence of such in the embryonic liver. It seems clear that more evidence is demanded. The 13 mm. human embryo is of the exact stage required. A careful study of its umbilical vesicle and the liver may be expected to give indication of the more probable conditions respecting primary hematopoiesis.

In anticipation of ensuing results it may be said that the evidence is all in favor of a continuous and identical hæmatogenetic process. And, in the sense that only one source of origin and only one line of cells can be recognized, the members of which appear identical in umbilical vesicle, liver, and heart, the evidence

supports the monophyletic theory of blood cell formation. Moreover, except for occasional endothelioblasts which become transformed into blood cells, the proliferating cells in the hepatic capillaries would seem to have been carried there by the blood currents. The verity of such a conclusion Schridde denies, claiming that the secondary are not daughter but sister cells of the primary erythroblasts.

The umbilical vesicle (fig. 1) here under consideration measured 6 mm. x 4 mm. It is almost spheric in shape. Externally it is faintly corrugated. Sections reveal a thicker wall distally and on one-half of its surface (fig. 2). These thicker regions contain the tubules and blood islands (fig. 3). The tubules are of two types; open and blind. The former are mostly cylindric in shape and generally open into the cavity of the vesicle by a constricted neck. The lining cells are similar to those lining the main cavity and continuous with them at the neck. The lumen is filled with an amorphous coagulum apparently identical with that of the cavity. Beyond the neck the tubules bend almost at right angles and generally pass distally, though occasionally proximally. The tubules sometimes branch once. Occasionally two limbs proceed from the neck, one proximally and one distally. The tubules are disposed parallel to the long axis of the vesicle. They vary in length from 100 to 200 microns.

Blind tubules result from the former type by occlusion of the neck followed by constriction and eventual separation from the lining epithelium. They appear cystic, are lined with more flattened epithelium and contain the same amorphous coagulum. None of these contain mesenchyme as recorded by Meyer. Moreover, the line of demarcation between entoderm and mesenchyme is here always distinct.

The entodermal cells lining the cavity of the vesicle vary from the cubic to the polyhedral type. The former is the prevailing type where the wall is thinnest. These cells have a deeply-staining almost homogeneous cytoplasm, and a centrally located nucleus. Distally and on one surface the lining epithelium is of the stratified polyhedral type (fig. 3). These cells contain a large centrally placed nucleus with one or several nucleoli and a reticulum with

occasional net-knots. The cytoplasm is greatly vacuolated and contains irregularly shaped flakes (cell detritus?) of deeper staining substances. Sometimes the nucleus appears suspended by several strands in the otherwise almost empty cell.

In the tubules similar types of cells occur: the flattened cells in cystic tubules or those with wide lumina, and the polyhedral cells (always in a single layer) in those with narrow lumina. In a general way the height of the epithelium varies inversely as the size of the lumen.

Besides the two types of tubules above described there are occasional solid cords of entodermal cells. These may be the results either of an original solid invagination of entoderm or of proliferation in a tubular structure.

The mesenchymal layer of the wall corresponds most closely to embryonic connective tissue. The predominating type of cell, however, is spindle-shaped and the whole structure is more compact and fibrillar. The amount of connective tissue in different regions varies inversely as the number of tubules. It contains everywhere blood vessels and capillaries. Distally the mesenchymal layer of the vesicle contains the blood islands.

The mesothelial covering (coelomic epithelium) is considerably more flattened than in the earlier vesicle (of a 9.2 mm. embryo) previously described. Neither in this nor in the younger vesicle could cilia be demonstrated on these cells as described by Branca.

The main points of difference between the two vesicles from the 9.2 mm. embryo and from the 13 mm. embryo respectively are as follows: (1) The older vesicle is slightly larger. (2) It contains fewer tubules (more of the blind variety and solid cords). (3) It contains blood-islands. (4) Its mesothelial cells are more flattened. (5) Its entodermal cells are more irregular and show signs of degeneration, *e.g.*, extreme vacuolization of protoplasm, absence of the "mucinous masses," and, in the cystic tubules and the more flattened cells of the vesicle, a disappearance of cell borders coupled with a decrease in size and staining capacity of the nuclei.

The above facts indicate that the human umbilical vesicle grows for a short time after the first month and while the entoderm

is undergoing the early phases of degeneration. The continued growth coincident with a degeneration of some of its elements (entodermal) indicates that the vesicle has a double function, viz., hæmatogenous, and some function of the entodermal cells.

That the umbilical vesicle subserves a hæmatogenous function in some degree has never been disputed. But this function is limited entirely to the mesenchyme. In the two specimens studied no evidence appears of an origin of giant cells (supposed blood mother-cells—Saxer) from the entoderm as held by Spee. Nor is the transition from entoderm to mesenchyme indistinct as described by Meyer. Moreover, the hæmatogenous function is commonly regarded as more or less incidental to a more primary function of the umbilical vesicle, especially as concerns its entodermal elements. The blood vessels are viewed as the purveyors of some sort of pabulum elaborated by the entodermal cells.

The several theories regarding the significance of the umbilical vesicle are: (1) that it has a hepatic function (Spee, Paladino, Saxer); (2) that it has an absorptive function like the intestine (Branca); (3) that it is a rudimentary or vestigial organ, "morphologically significant, but functionally nil" (Selenka); (4) that it has primarily a hæmatopoietic function (Hubrecht, Bonnet). A hepatic significance is urged on the basis (1) of a structural resemblance between liver and umbilical vesicle (Spee and Saxer); (2) of the presence of glycogen (Paladino); (3) of the presence in both of giant-cells—(Spee). This hypothesis is invalidated by the following facts: (1) The resemblance between liver and the wall of the umbilical vesicle is only general, not detailed (Branca). (2) Giant cells are found at this stage, also in the mesonephros and the heart. (3) Most embryonic tissues contain glycogen (Gage).

A nutritive significance is urged by Branca on these grounds: (1) Supposed presence of small amount of yolk in the human "yolk sac." (2) Morphologic similarity between the lining cells of the sac and those of the small intestine. The details include (a) terminal bars, (b) ciliated borders, (c) position of nucleus, and (d) cell contents, which he likens to ergastoplasm (prozymogen) and zymogen. (3) Common origin of the intestine and yolk sac from primary entoderm. To these might be added Bonnet's

contention that yolk must needs be present to supply hæmoglobin for the first erythroblasts.

Branca regards the entodermal cells as agents for the preparation and transference in usable form of yolk contained in the vesicle and needed as food by the embryo. This hypothesis appears untenable for the following reasons: (1) The presence of yolk is not established. (2) Common origin need not imply identity of function, *e.g.*, cells of villi (absorptive) and chief cells (secretory) of fundus glands. The umbilical cells may functionally resemble more closely the chief cells. (3) Absence of ergastoplasmic granules in cells of absorption. (4) On the basis of mere staining reaction these masses of granules may with equally good reason be regarded as "mucinous masses." (5) The presence of ciliated borders on some of the cells vitiates a strict homology. (6) The flakes and granules in the distal portions of the cells are too large and irregular to be regarded as zymogen granules. (7) The structural peculiarities, even as interpreted by Branca, are more like those of secretory cells. (8) The hæmoglobin may have the same source as the lipid and glycogen content of the cell.

Branca, moreover, urges in support of a nutritive function that the umbilical vesicle cannot be considered as a merely rudimentary structure since a decrease in size involves an atrophy of the constituent elements as in the case of the epiphysis and the notochord. But an organ in becoming rudimentary need not necessarily decrease via an atrophy, but only a decrease in number, of its elements, *e.g.*, vermiform appendix. Again, an organ may become rudimentary in part and still retain an important collateral function, *e.g.*, hypophysis.

The human umbilical vesicle would seem to have lost its function of yolk absorption and elaboration, but to have retained the very important coincident function of hæmatopoiesis. When the liver takes up the work of blood cell formation, the umbilical vesicle decreases in size by reason of an atrophy of its elements and eventually disappears. It is not simply a vestigial structure, but appears to have a necessary function in supplying the progenitors of the foetal blood cells. Accordingly it must be regarded as the earliest hæmatopoietic organ.

Furthermore, the complicated histologic structure due to the presence of the entodermal tubules must be interpreted in the light of the phylogenetic history of the mammals. In sauropsidan ancestors with meroblastic yolk-laden eggs, the entodermal cells functionated in the elaboration and absorption of the yolk. This process involved the initial secretion of a liquifying fluid. Thus prepared in soluble form yolk was transferred to the blood vessels. The entoderm seems to have retained its secretory function but

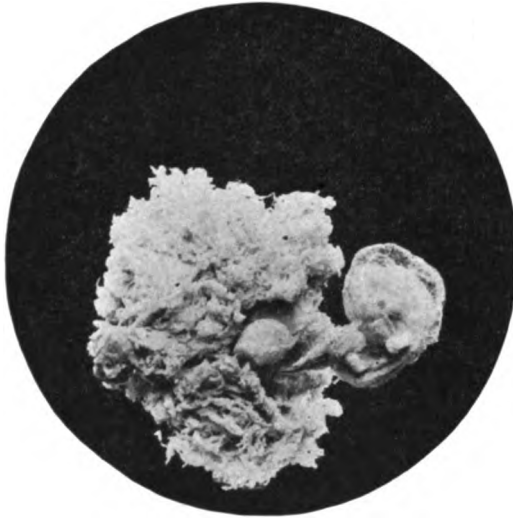


FIG. 1. Photograph of opened chorionic vesicle of 13 mm. human embryo, showing umbilical vesicle and amnion intact,  $\times \frac{1}{2}$ . Made by Mr. Frank P. Smart, University of Virginia.

there is no yolk to liquefy and absorb. The amorphous coagulum in the tubules and cavity is perhaps the representative of this yolk-dissolving secretion. That a fluid is actually secreted by the tubules is the more probable when one considers the varying character of the lining cells. This is best interpreted in terms of pressure exerted by a liquid content of the tubules. The apparently gratuitous extension of the entoderm seems due to hereditary factors consequent upon a sauropsidan ancestry.

On the basis of very many facts of comparative anatomy, Hubrecht argues cogently for the primitive character of the Primates in many respects (yolk sac, allantois, etc.), and against a sauropsidan ancestry of the mammals. The structure of the umbilical vesicle, as regards more particularly its tubules and blood islands accords better with the assumption of a sauropsidan descent. The evidence suggests more forcibly a secondary modification, along the lines of greater hematopoietic significance, of a formerly predominantly nutritive organ.

Hubrecht also emphasizes the hæmatopoietic significance of the yolk sac chiefly on the grounds, (1) that the liver during early stages cannot be said to be capable of supplying a sufficient number of blood cells for assisting in metabolic processes, and (2) that due to the presence of a decidua capsularis no nutritive material can enter the extra embryonic coelom to be transported by the blood vessels to the embryo. In the absence of yolk the sole purpose of the vessels is to produce (in the first instance) and transport blood cells to the embryo. But these facts are urged in favor of its primitive character. The peculiar characters of the entoderm, however, seem more intelligible as secondary modifications of primitive sauropsidan conditions.

It remains to describe the blood islands. They arise in the mesenchyme exactly as described for a number of birds and mammals: Portions of the syncytial mesenchyme become transformed into irregular cords of cells, the peripheral cells of which form the endothelial wall, the central cells blood corpuscles (fig. 4). The only detailed study of blood islands in the human umbilical vesicle previously made, as far as I can learn, is that of Schridde. But he describes "Bluträume" as the original structures. Only subsequently do the first blood cells arise from cells of the vessel-wall. The evidence from a study of the blood islands in my specimen is unequivocally opposed to this procedure. Moreover, the details of early hæmatogenesis are exactly similar to those described by Maximow for rabbit, guinea pig, cat, rat and dog. Among the central cells of a blood island are seen three successive stages yielding three distinct types of cells: (a) lymphocyte, (b) megalo-blast, and (c) normoblast. All these cells can be seen in mitosis,

the first two types more abundantly. Occasionally a cell of the vessel-wall is seen to round up and become free as a lymphocyte, as described by Schridde for all the cells.

The first lymphocytes have a light-staining granular cytoplasm and a kidney-shaped nucleus with nucleoli and karyosomes. The

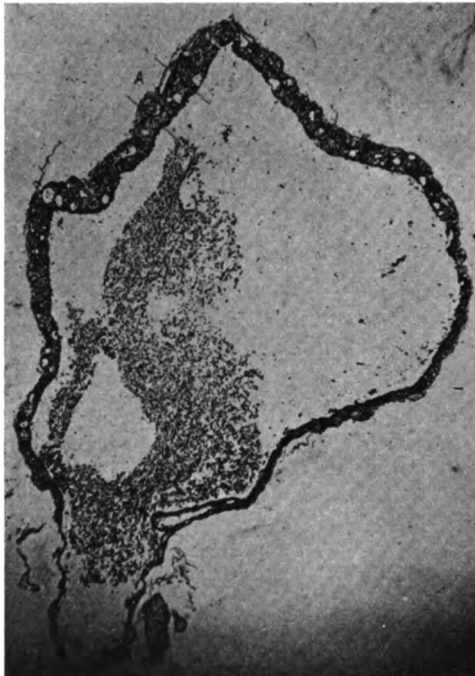


FIG. 2. Photomicrograph of a transverse section of the umbilical vesicle near the mid-region, showing the character of the wall and the content of the vesicle  $\times 30$ . Reduced  $\frac{1}{2}$  in reproduction. Made by Dr. Leopold Jaches, Cornell University Medical College, New York City.

later stages and generations of these have large, round and more strongly basophile nuclei, also with nucleoli and karyosomes and a narrow shell of basophile cytoplasm. These cells are actively amœboid as seen by their pseudopodia. They are the progenitors of both other cells like themselves (lymphocytes) and red blood cells.



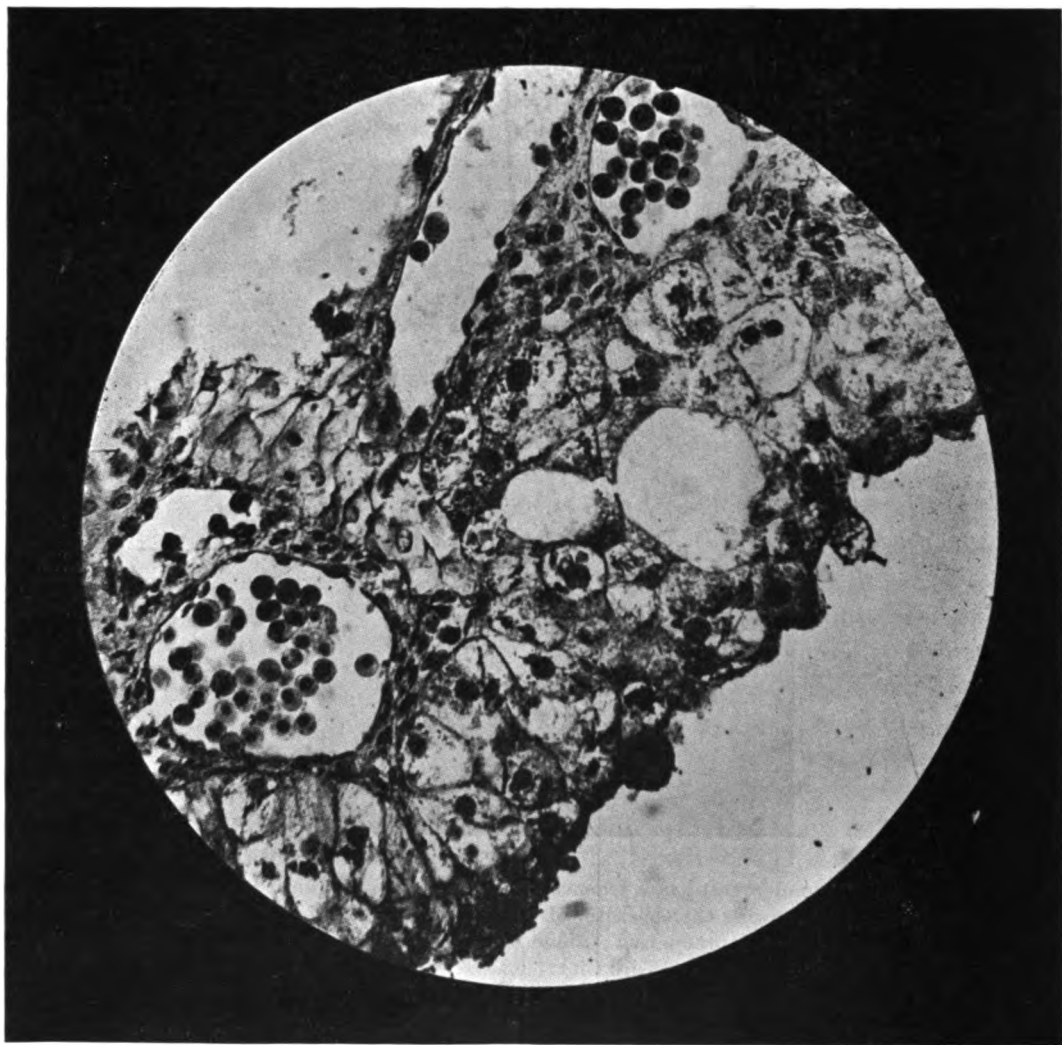


FIG. 3. Photomicrograph of region (A) of fig. 2, more highly magnified, showing a branching tubule, and a small blood island (in the angle between the blood vessels in the upper part of the illustration)  $\times 240$ . Made by Dr. Leopold Jaches.

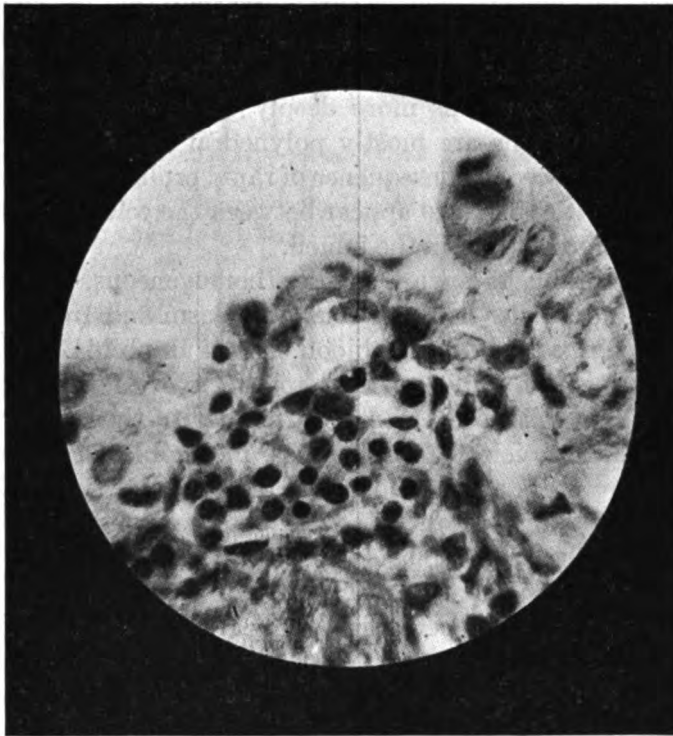


FIG. 4. Photomicrograph of large blood island. The upper row of cells shows several types of lymphocytes. The majority of the remaining cells are normoblasts. The large lighter staining cell to the left of the center of the island is a megaloblast,  $\times 350$ . Made by Mr. Frank P. Smart.

The megaloblasts contain smaller, lighter-staining, spheric nuclei, and have a great amount of light-staining granular cytoplasm. The earlier generations of these are the largest cells seen (2 to 3 times the size of post-foetal erythrocytes).

The normoblasts are smaller both as respects the nucleus and the cells. The nucleus is more chromatic than that of the megaloblast. It also contains nucleoli and a reticulum with net-knots. The cytoplasm is homogeneous, probably due to the presence of hæmoglobin, and stains more deeply than that of the megaloblasts. These cells are mostly polyhedral in shape due to the crowding produced in consequence of rapid proliferation of megaloblasts. Cavities begin to appear between the cells, and the latter subsequently float free in the lumen.

The erythroblasts have a smaller, homogeneous, pale-staining nucleus; and a paler homogeneous cytoplasm and frequently an oval shape, recalling the red blood cells of amphibia.

When attention is now turned to the liver of this specimen, one sees here cells in every respect similar to those described in the umbilical vesicle. Lymphocytes, megaloblasts, normoblasts and erythroblasts appear, identical in form and size. The relative number only varies. The lymphocytes and megaloblasts are rarer. The erythroblasts are more abundant. The normoblasts greatly preponderate. The latter are actively proliferating. Only rarely is a blood cell seen arising from the endothelial wall of the hepatic capillaries. Since no extravascular masses of proliferating blood cells, as described by Schridde for a 13 mm. embryo, appear in this specimen, and only very rarely an extravascular lymphocyte, the presumption is strong that the blood cells of the liver and heart have been carried there by the current from the umbilical vesicle. Here they find favorable harbors for continued proliferation. There appears no evidence to furnish ground for dividing hæmatopoietic phenomena in the first weeks of human development into the two stages described by Schridde.

Concerning the method of enucleation of erythroblasts in the formation of erythrocytes whether by intracellular absorption (Schridde) or by extrusion (Howell and Maximow) nothing can be decided here. Obviously also no observations can be made

regarding the origin of leucocytes and small lymphocytes since these do not yet appear. The evidence, however, as far as it goes agrees with Maximow's findings for the early stages in the guinea pig, etc., and to this extent accords with the monophyletic theory of blood cell formation.

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## A LIST OF NORMAL HUMAN EMBRYOS WHICH HAVE BEEN CUT INTO SERIAL SECTIONS

FRANKLIN P. MALL

*From the Anatomical Laboratory, Johns Hopkins University*

At the Baltimore meeting of the Anatomists a number of embryologists requested The Wistar Institute to prepare a catalogue of human embryos found in various laboratories, museums and private collections in the United States, which have been sectioned and mounted for study. Accordingly a blank was prepared which was widely distributed, but replies were obtained from only those laboratories which possessed a number of series of sections. These blanks, which were not uniformly filled out, were given to me by Dr. Greenman, with the request that they be blended into a single list for the use of those interested in human embryology. Before publishing it, Dr. Huber kindly inserted a note in *THE ANATOMICAL RECORD* asking for further data, especially concerning specimens which had not yet been reported upon. The replies thus received, together with those secured by Dr. Greenman, make this list as complete as we can hope to make it at the present time.

There are about 300 specimens listed in this catalogue, and of these about one-half are well preserved. When it is considered that the series of human embryos in the embryological collection of the Anatomical Laboratory of Johns Hopkins University are selected from about 1000 abortions, it may safely be stated that but 5 per cent of them yield good serial sections of normal embryos. This list then is a selected one from about 3000 abortions,—ova, embryos and young foetuses.

As the embryos in a collection are usually referred to by the name of the collector, this designation has been retained in this list. By consulting therefore the list of the names of anatomists as given in this volume of *THE ANATOMICAL RECORD* it will be easy to learn where an embryo of a given length may be found.

The specimens are arranged according to their length. The medium in which they were measured is also given. The number of slides in the series is of value when requests for loans are made. The "remarks" are self explanatory. Only the chief stain is given in each case. Most of the embryos are counterstained; in Minot's collection often with Lyons blue, in Jackson's with congo red, in Gage's with eosin; and in Huber's and mine, some with eosin and some with congo red.

The specimens are arranged first, according to their crown-rump length, then according to the direction of the sections, and finally according to the quality of the series. Only those marked "excellent" or "good" are of great value for study, but in special cases "fair" and "poor" series no doubt will be of value.

At a future date it may be desirable to rearrange the specimens with profile outlines of each stage, and this would be a great step in advance, for undoubtedly embryos several millimeters apart in this list may be of the same stage of development. Sooner or later we must designate embryos by their stage of development, not by their crown-rump length,—certainly not by their age, which is the worst method of all and has brought about much confusion in embryology.

The use of a list as here given is amply justified when we consider the nature of the subject before us. Only by coöperation can we hope to advance the science of human embryology,—a subject which is of first importance to anatomists. Witness the value of coöperation in His's "*Anatomie menschlicher Embryonen*" and in Keibel and Elze's "*Normentafel zur Entwicklungsgeschichte des Menschen*." His secured embryos through the aid of various physicians and scientists, but it took a lifetime for him to make a fairly satisfactory collection of his own, as his various publications

will show. Keibel and Elze borrowed embryos from different European collections and thereby were placed at an advantage. These two works are monumental.

A selected collection of pictures of human embryos which includes several of this list is given in Kollmann's "*Handatlas der Entwicklungsgeschichte des Menschen*." At present there is in preparation a larger work on Human Embryology, in two volumes, which will be published shortly both in Leipzig and Philadelphia. About half of this treatise is being prepared by Americans and is based largely upon the collections represented in this list. This coöperative undertaking is not supported by any research grant, but those who have endowed this work with their energies know only too well that their powers would have been greatly augmented had such support been given. It may here be emphasized that efforts to promote the science of human embryology have not received financial support excepting the work of Keibel and Elze, for which they received grants from the Grand Duchy of Baden and from the Royal Prussian Academy of Sciences.

The difficulty of obtaining good material, the care and time consumed in the preparation of good series and in the study of the same (involving often the necessity of making time-consuming reconstructions) makes progress in the study of human embryology very slow. During the past 35 years His repeatedly called attention to the need of financial endowment for, and coöperation in the study of human embryology. At the first meeting of the International Association of Academies, held in Paris in 1901, His proposed that commissions be appointed for the promotion of the study of human embryology and neurology. At that meeting we saw the birth of the Brain Commission, which is working effectively for the study of the brain. A series of Interacademic Institutes has been established, including our Wistar Institute. At that time the International Association of Academies recommended that the study of human embryology should be supported by various anatomical societies. No steps in this direction were, however, taken by the First International Anatomical Congress, which convened four years later at Geneva. Possibly the



Second Congress, which meets this summer in Brussels, may be induced to consider the matter.

These statements indicate that anatomists as individuals are coöperating, but their work will progress slowly unless institutes be established to carry it on with greater vigor. The problem is a clear one. It is full of promise. Human embryology is as important and merits as careful investigation as does the field of neurology. Until the human embryo has been thoroughly investigated human anatomy will not rest upon a sound basis.

COLLECTION	NUMBER OF EMBRYO	NUMBER OF SLIDES IN THE SERIES	CROWN RUMP LENGTH OF THE EMBRYO IN MILLIMETERS	IN WHAT MEDIUM MEASURED	REMARKS	DIRECTION OF SECTION T=TRANSVERSE S=SAGITTAL C=CORONAL	THICKNESS OF SECTIONS IN $\mu$	PRINCIPAL STAIN H=HEMATOXYLIN C=CARMINE	CONDITION OF TISSUE E=EXCELLENT F=FAIR G=GOOD P=POOR	
Huber	39	68	....	Formalin	Ovum	..	7	H	G	
Mall	134	3	....	.....	Ovum	..	..	H	G	
Mall	278	10	....	Formalin	Ovum	..	20	C	G	
Minot	825	5	....	.....	Ovum	..	8	C	E	
Minot	1500	25	1.54	Slide	Ovum	T	7	H	G	
Minot	1586	10	....	.....	Ovum	..	10	Mixed	.....	
Huber	43	..	....	Alcohol	Ovum	..	..	..	P	
Mall	13	2	1.4	.....	Ovum	..	10	H	F	
Huber	37	3	1.5(?)	Alcohol	.....	C	10	H	P	
Mall	3	6	2.5	.....	.....	T	10	H	G	
Mall	12	6	2.1	Alcohol	.....	T	10	C	G	
Mall	318	8	2.5	.....	.....	T	25	C	G	
Mall	391	17	2	Formalin	.....	T	10	C	G	
McMurrich	11	4	2.5	.....	Incomplete	T	10	H	G	
Mall	384	11	2	Formalin	.....	T	10	C	F	
Stockard	0	3	2.2	On slide	.....	S	8	H	E	
Mall	250	11	2	Formalin	.....	S	20	C	F	
Huber	24	3	2.1	Alcohol	.....	C	10	H	F	
Huber	12	1	2.4	Formalin	.....	C	20	H	P	
Mall	186	4	3.5	.....	.....	T	20	C	G	
McMurrich	5	3	3	Alcohol	Twins	{	T	10	H	F
McMurrich	6	4	3	Alcohol			T	10	H	F
Mall	164	4	3.5	Formalin	.....	T	20	C	F	
Mall	209	5	3	Alcohol	.....	T	50	C	F	

COLLECTION	NUMBER OF EMBRYO	NUMBER OF SLIDES IN THE SERIES	CROWN RUMP LENGTH OF THE EMBRYO IN MILLIMETERS	IN WHAT MEDIUM MEASURED	REMARKS	DIRECTION OF SECTION T=TRANSVERSE S=SAGITTAL C=CORONAL	THICKNESS OF SECTIONS IN $\mu$	PRINCIPAL STAIN H=HEMATOXYLIN C=CARMINE	CONDITION OF TISSUE E=EXCELLENT F=FAIR G=GOOD P=POOR
Mall	239	11	3	Formalin	.....	T	10	H	F
Huber	1	6	3	Alcohol	.....	S	5	H	P
Huber	45	2	3	Formalin	.....	S	10	H	P
Huber	13	1	3.5	Formalin	.....	C	20	H	P
Huber	44	4	3.5	Formalin	.....	C	10	H	P
Mall	148	7	4.3	Alcohol	.....	T	10	H	G
Mall	76	10	4.5	Slide	.....	T	20	C	G
Minot	714	2	4.0	Formalin	.....	T	6	C	E
Mall	87	10	4	Alcohol	.....	T	20	C	F
Mall	1	4	4.5	Alcohol	.....	T	10	C	P
Mall	248	2	4.5	.....	.....	T	50	C	P
Mall	112	6	4	Slide	.....	S	10	H	P
Jordan	1	19	5	Fresh	.....	C	10	H	E
			4	On Slide	.....				
Mall	136	15	4	Alcohol	.....	C	50	C	
Huber	19	4	4	Formalin	.....	C	10	H	P
Huber	36	5	4	Formalin	.....	C	10	H	F
Huber	40	4	4	Formalin	.....	C	10	H	P
Greenman	15009	4	4	Formalin	.....	C	10	H	P
Mall	80	15	5	Alcohol	.....	T	20	C	G
Mall	19	11	5.5	Alcohol	.....	T	20	C	F
Gage	5	48	5	.....	.....	S	20	H	E
Mall	116	6	5	Formalin	.....	S	20	C	G
Huber	8	12	5	Formalin	.....	C	5	H	P
Huber	9	13	5	Formalin	.....	C	5	C	P
Mall	241	7	6	Formalin	.....	T	10	H	G
Mall	245	53	6	Zenker	.....	T	5	H	F
Jackson	176	15	6	Fresh	.....	T	10	H	P
Mall	371	8	6.6	Formalin	.....	S	10	H	G
Mall	2	12	7	Alcohol	.....	T	15	C	G
Mall	372	16	7	.....	.....	T	10	H	G
Mall	383	13	7	.....	.....	T	10	H	G
McMurrich	3	7	7.5	Alcohol	.....	T	15	C	G
Mall	4	14	7	Alcohol	.....	T	10	C	F
Mall	18	33	7	Alcohol	.....	T	20	C	F
Jackson	220	26	7.3	Alcohol	.....	T	10	H	P
Huber	51	8	7.5	Formalin	.....	S	10	H	G
Mall	221	6	7.5	Slide	.....	S	20	C	G

COLLECTION	NUMBER OF EMBRYO	NUMBER OF SLIDES IN THE SERIES	CROWN RUMP LENGTH OF THE EMBRYO IN MILLIMETERS	IN WHAT MEDIUM MEASURED	REMARKS	DIRECTION OF SECTION T=TRANSVERSE S=SAGITTAL C=CORONAL	THICKNESS OF SECTIONS IN $\mu$	PRINCIPAL STAIN H=HEMATOXYLIN C=CARME	CONDITION OF TISSUE E=EXCELLENT F=FAIR G=GOOD P=POOR
Mall	380	5	7.5	Alcohol	.....	S	..	H	F
Mall	187	4	7	Alcohol	.....	S	20	C	P
Mall	208	5	7	Alcohol	.....	S	20	C	P
Minot	256	7	7.5	Alcohol	.....	C	10	C	F
Huber	21	15	7.5	Formalin	.....	C	10	H	F
Huber	10	6	7	Formalin	.....	C	20	H	P
Stockard	9	17	8.5	Alcohol	.....	T	10	H	E
Mall	397	12	8	Formalin	.....	T	10	H	G
Mall	387	10	8	Alcohol	.....	T	20	H	F
			11	Formalin	.....				
Mall	88	9	8	Alcohol	.....	T	50 and 100	C	P
							20		
Mall	289	10	8	Alcohol	.....	T	20	H	P
Mall	389	7	8	Alcohol	.....	S	20	H	G
			10	Formalin	.....				
Minot	817	5	8	Alcohol	.....	C	10	C	E
Minot	818	2	8	Alcohol	Yolk sac	S	10	C	E
Mall	113	16	8	Slide	.....	C	10	C	F
Huber	11	9	8	Formalin	.....	C	15	H	P
Stockard	10	81	9.2	Alcohol	.....	T	10	H	G
Mall	163	11	9	Formalin	.....	T	20	C	G
Minot	523	11	9.4	Alcohol	.....	T	10	C	G
Minot	734	9	9.2	Alcohol	.....	T	10	C	E
Minot	1005	9	9.4	Alcohol	.....	T	8	C	P
Mall	422	4	9	Clearing	.....	S	40	C	G
				fluid	.....				
Minot	1002	3	9.6	Formalin	.....	S	8	C	F
Mall	388	14	9	Formalin	.....	S	25	C	P
Mall	452	5	9	Formalin	.....	S	40	C	P
Minot	1001	5	9.6	Formalin	.....	C	8	C	G
Mall	352	37	9	.....	.....	C	10	H	F
							15		
Huber	35	20	9	Formalin	.....	C	{10 15}	H	P
Jordan	2	62	13	Fresh	.....	T	10	C	E
			10.5	On slide					
Mall	109	27	10.5	Alcohol	.....	T	20	C	G
Minot	1000	13	10.0	Alcohol	.....	T	6	C	E

COLLECTION	NUMBER OF EMBRYO		NUMBER OF SLIDES IN THE SERIES	CROWN RUMP LENGTH OF THE EMBRYO IN MILLIMETERS	IN WHAT MEDIUM MEASURED	REMARKS	DIRECTION OF SECTION T=TRANSVERSE S=SAGITTAL C=CORONAL	THICKNESS OF SECTIONS IN $\mu$	PRINCIPAL STAIN H=HEMATOXYLIN C=CARMINE	CONDITION OF TISSUE E=EXCELLENT F=FAIR G=GOOD P=POOR
Reese	17	10	10				T	20	H	P
Huber	3	30	10		Alcohol		S	5	H	E
Minot	736	9	10.2		Alcohol		S	10	C	G
Mall	114	26	10				S	10	C	F
Huber	2	34	10		Formalin		S	5	H	P
Jackson	60	35	11		Alcohol		T	20	C	G
Kingsbury	25	56	11				T	10	C	G
Minot	189	15	11.5		Alcohol		T	15	C	E
Minot	1605	5	11.7			Broken	T	10	H	E
Mall	231	6	12		Alcohol		T	100	C	P
Minot	1006	15	11.5		Formalin		S	8	C	P
Mall	353	34	11				C	10	H	G
Minot	816	11	12.0		Alcohol		C	10	C	E
Stockard	3	49	12		Alcohol		T	10	H	G
Gage	6	99	12				T	10	C	G
Jackson	134	20	12		Formalin		T	20	C	P
Mall	447	13	12	Alcohol	}		S	20	H	F
			12	Formalin						
Gage	7	89	12.5				S	15	H	P
Mall	317	26	12.5		Formalin		C	20	H	G
Mall	351	4	12			Injected	C	250		G
Huber	16	20	12.5		Formalin		C	10	H	F
Kingsbury	26	50	13				T	10	H	E
Mall	175	16	13		Alcohol		T	20	C	G
Minot	839	23	13.6		Alcohol		T	10	C	E
Mall	406	15	13	Alcohol	}		S	20	H	G
			16	Formalin						
Huber	52	23	13.5		Formalin		C	10	H	G
Greenman	15115	23	13.5		Formalin		C	10	H	G
Stockard	5	67	14		Alcohol		T	10	H	E
Minot	1003	20	14.5		Formalin		T	10	C	F
Minot	1004	3	14.5		Formalin		T	10	C	F
Jackson	97	25	14		Fresh		T	20	C	P
Reese	18	25	14				T	20	H	P
Mall	144	13	14		Slide		S	40	C	G
Mall	423	11	15.2	Clearing	}		T	50	C	G
			fluid							
Huber	4	32	15		Müller's		S	10	H	G

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Mall	390	24	15.5	Formalin	Injected	S	20—50	C	G
Mall	350	89	15	Formalin	.....	G	10	H	G
Greenman	15116	14	15	Formalin	.....	G	10—25	H	F
Gage	12	116	15	.....	.....	C	10	H	F
Huber	53	14	15	Formalin	.....	C	10—25	H	F
Mall	74	30	16	Slide	.....	T	50	C	G
Mall	409	28	16	Alcohol	.....	T	20	H	G
		18	Formalin						
Minot	1322	32	16.0	Alcohol	.....	T	8	C	G
Huber	6	50	16	Formalin	.....	S	10	H	G
Mall	43	21	16	Alcohol	.....	S	50	C	G
Jackson	133	51	16	Formalin	.....	S	20	C	F
Mall	256	17	16	Alcohol	.....	S	25 and 50	C	F
Minot	1128	24	16.0	Formalin	.....	C	14	C	F
Mall	106	23	17	Fresh	.....	T	50	C	G
Mall	296	58	17	.....	.....	T	20	Mixed	G
Mall	424	11	17.2	Alcohol	Injected	T	50	C	G
Jackson	58	38	17	Fresh	.....	T	20	C	G
Mall	9	25	17.5	Alcohol	.....	T	20	C	F
Mall	404	15	17	Alcohol	.....	T	40	C	P
Mall	216	16	17	Alcohol	.....	T	50 and 100	C	P
Mall	8	7	17	Alcohol	.....	T	40	C	P
Bardeen	2	30	17	On Slide	.....	S	20	C	G
Huber	14	45	17	Formalin	.....	C	10	H	G
Mall	5	49	18.5	Alcohol	.....	T	20	C	F
Mall	17	23	18	Alcohol	.....	T	50 and 100	C	P
Bardeen	1	37	18	On slide	.....	S	20	C	G
Mall	431	46	18.5	Alcohol	.....	S	20	H	G
		19	Formalin						
Mall	432	43	18	Alcohol	.....	S	20	H	G
		18.5	Formalin						
Huber	5	50	18	Formalin	.....	S	10	H	G

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Minot	1129	28	18.1	Formalin	.....	S	14	C	P
Mall	338	12	18	Alcohol	.....	S	25 and 50	C	P
Huber	45	69	18.5	Formalin	.....	C	10	H	F
Jackson	98	82	18	Fresh	.....	C	26	C	F
Greenman	15026	69	18.5	Formalin	.....	C	10	H	F
Gage	3	86	19	.....	.....	T	20	C	E
Minot	819	32	19.0	Alcohol	.....	T	14	C	F
Minot	1597	..	19.3	Formalin	.....	T	12	C	E
Mall	7	9	19.5	Alcohol	.....	T	40	C	P
Huber	32	40	19	Formalin	.....	S	15	H	G
Mall	229	22	19	Alcohol	.....	S	50	C	G
Greenman	14597	24	19	Fresh	.....	C	100	C	G
Mall	293	19	19	Formalin	.....	S	50	C	F
Greenman	15010	39	19	Formalin	.....	C	10	H	F
Minot	828	39	19.0	Alcohol	.....	C	12	C	P
Huber	41	39	19	Formalin	.....	C	10	H	F
Mall	22	40	20	Alcohol	.....	T	50	C	G
Mall	453	40	20 23	Alcohol Formalin	.....	S	20	H	F
Mall	368	34	20	.....	.....	S	20	H	P
Mall	128	37	20	Formalin	.....	C	50	C	G
Mall	240	69	20	Formalin	.....	C	20	H	G
Mall	349	7	20	Formalin	Injected	C	250	None	G
McMurrich	19	20	20	Formalin	Head	C	20	H	F
Minot	744	65	21.0	Alcohol	.....	T	14	C	P
Minot	852	..	21.8	Alcohol	Head	S	10	H	..
Stockard	1	163	22	Alcohol	.....	T	10	H	E
Conklin	1	77	22	On slide	.....	T	64	H	G
McMurrich	1	62	22	Alcohol	.....	T	15	C	G
Minot	871	59	22.8	Alcohol	.....	T	12	C	F
Kingsbury	27	95	22	.....	.....	T	15	C	F
Stockard	6	208	22	Alcohol	.....	S	10	H	G
Minot	851	120	22.0	Alcohol	.....	S	12	C	G
Mall	108	..	22	Slide	.....	S	45	C	F
Huber	7	80	22	Formalin	.....	S	15	H	P

COLLECTION	NUMBER OF EMBRYO		CROWN RUMP LENGTH OF THE EMBRYO IN MILLIMETERS	IN WHAT MEDIUM MEASURED	REMARKS	DIRECTION OF SECTION T=TRANSVERSE S=SAGITTAL C=CORONAL	THICKNESS OF SECTIONS IN $\mu$	PRINCIPAL STAIN H=HEMATOXYLIN C=CARME	CONDITION OF TISSUE E=EXCELLENT F=FAIR G=GOOD P=POOR
	NUMBER OF SLIDES IN THE SERIES								
Mall	268	34	22	Formalin	.....	S	25 and 50	C	P
Minot	737	100	22.8	Alcohol	.....	C	12	C	E
Minot	192	6	23.0	Alcohol	Yolk sac	T	13	C	F
Mall	242	58	23	Alcohol	Double	T	40	C	F
Mall	382	29	23	.....	Injected	S	50	C	G
Minot	181	68	23.0	Alcohol	.....	S	20	C	E
Gage	9	80	23	.....	.....	S	20	H	F
Jackson	147	80	23	Alcohol	.....	S	20	C	F
Stockard	2	206	23	Alcohol	.....	S	10	H	F
Huber	38	45	23	Alcohol	.....	S	15	H	F
Mall	57	40	23	Alcohol	.....	S	50	C	P
Jackson	56	117	24	Alcohol	.....	T	20	C	G
Mall	6	38	24	Alcohol	.....	T	40	C	G
Mall	405	31	24	Alcohol	.....	S	40	C	G
.....	.....	.....	26	Formalin	.....	.....	.....	.....	.....
Minot	24	29	24.0	Alcohol	Head	C	20	C	G
Minot	38	34	24.0	Alcohol	Trunk	T	20	C	G
Mall	10	19	24	Alcohol	.....	T	40	C	F
Mall	314	28	24	Alcohol	.....	S	50	C	F
Kingsbury	28	88	25.6	.....	.....	T	15	H	G
Kingsbury	29	86	25	On slide	.....	T	15	H	F
Huber	33	50	25	Alcohol	.....	S	15	H	F
McMurrich	2	46	26	Alcohol	.....	T	20	C	G
Jackson	99	199	26	Fresh	.....	T	20	C	P
Mall	100	96	27	Alcohol	.....	S	50	C	P
Mall	45	60	28	Alcohol	.....	T	50	C	G
Minot	1598	.....	28.8	Formalin	.....	S	12	C	E
Huber	49	108	29	Formalin	.....	C	15	H	G
Minot	914	121	29.0	Alcohol	.....	C	20	C	G
Greenman	15024	108	29	Formalin	.....	C	15	H	G
Minot	913	88	30.0	Alcohol	.....	T	14	C	G
Mall	75	105	30	Alcohol	.....	S	50	C	G
.....	.....	.....	.....	.....	.....	.....	50 and 100	.....	.....
Mall	227	39	30	.....	.....	S	.....	C	G
Huber	15	120	30	Formalin	.....	C	20	H	G

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	NUMBER OF SLIDES IN THE SERIES								
Mall	86	115	30	Formalin	.....	C	50	C	G
Jackson	57	262	31	Fresh	.....	T	20	C	G
Huber	46	169	31	Formalin	.....	S	15	H	E
Gage	11	275	31	.....	.....	C	20	H	G
Minot	648	25	32.0	Alcohol	Head	T	..	C	F
Minot	649	81	32.0	Alcohol	Trunk	T	..	C	F
Minot	290	42	32.0	Alcohol	Head	S	20	C	F
Minot	292	113	32.0	Alcohol	.....	S	24	C	F
Minot	291	53	32.0	Alcohol	Head	C	20	C	F
Mall	211	44	33	Formalin	.....	S	33	C	G
Gage	19	666	34	.....	.....	T	15	H	G
Mall	449	103	34	Alcohol	Injected	S	{ 10 and 100 }	H	G
Mall	199	64	35	.....	Incomplete	S	50	H	G
Huber	22	35	35	Alcohol	.....	S	10	H	F
Jackson	51	46	35	Formalin	.....	S	{ 50 and 200 }	C	F
Gage	4	470	36	.....	.....	S	20	H	F
Minot	820	264	37.0	Alcohol	.....	T	14	C	F
Mall	249	41	37	Alcohol	Double	T	50	C	F
Mall	145	34	38	Formalin	.....	S	{ 50 and 100 }	C	G
Huber	17	210	39	Formalin	.....	C&S	10	H	E
Jackson	122	100	39	Formalin	.....	T	60	C	F
Mall	224	74	40	Formalin	.....	S	{ 50 and 100 }	C	G
McMurrich	4	46	40	Formalin	Head	C	40	H	G
Minot	838	201	42.0	Alcohol	Trunk	T	14	C	F
Minot	841	161	42.0	Alcohol	Head	T	14	C	F
Minot	1611	....	44.3	Alcohol	.....	C&S	14	C	G
Huber	18	300	45	Formalin	.....	C&S	10	H	E
Mall	95	86	46	Formalin	.....	S	100	C	G
Jackson	121	100	46	Formalin	.....	T	60	C	F
Huber	48	189	47	Formalin	.....	S	15	H	G



COLLECTION	NUMBER OF EMBRYO	NUMBER OF SLIDES IN THE SERIES	CROWN RUMP LENGTH OF THE EMBRYO IN MILLIMETERS	IN WHAT MEDIUM MEASURED	REMARKS	DIRECTION OF SECTIONS T=TRANSVERSE S=SAGITTAL C=CORONAL	THICKNESS OF SECTIONS IN $\mu$	PRINCIPAL STAIN H=HEMATOXYLIN C=CARMINE	CONDITION OF TISSUE E=EXCELLENT F=FAIR G=GOOD P=POOR
Huber	47	248	48	Formalin	.....	S	20	H	F
Mall	84	217	50	Alcohol	.....	T	50	C	G
Gage	10	116	50	.....	.....	T	15	H	G
Brödel	3	..	50	.....	Injected	T	..	C	G
Mall	96	129	50	Formalin	.....	S	100	C	G
Mall	184	108	50	Formalin	.....	S	50 and 100	C	G
Jackson	115	337	50	Fresh	.....	S	40	C	F
Gage	15	280	53	.....	.....	S	15	H	G
Mall	267	166	59	Formalin	Head	S	20	Mal- lory	G
Huber	23	400	60	Formalin	.....	C&T	10	H	G
Brödel	1	..	60	.....	Injected	T	..	..	G
Mall	306a	43	60	Formalin	Hands	S	25	C	G
Jackson	55	218	65	Formalin	Trunk	T	50	C	F
Jackson	55	200	65	Formalin	Head	T	45	C	F
Minot	722	56	78	Alcohol	Head	T	30 and 37	..	G
Minot	723	58	78	Alcohol	Thorax	T	30 and 45	..	G
Minot	724	19	78	Alcohol	Abdomen	T	..	..	G
Minot	725	16	78	Alcohol	Abdomen	T	..	..	G
Minot	728	7	78	Alcohol	Penis	S	15	C	G
Minot	729	3	78	Alcohol	Finger	S	13	C	G
Minot	730	6	78	Alcohol	Toe	S	..	C	G
Mall	44	56	70	Alcohol	Trunk	T	100	C	G
Mall	23	59	70	Alcohol	Trunk	T	100	C	G
Mall	179	32	70	Formalin	Trunk	T	50	C	P
Minot	720	34	78	Alcohol	Face	C	30 and 37	..	G
Minot	721	19	78	Alcohol	Head	C	37	..	G
Mall	34	60	80	Alcohol	Trunk	T	50	C	G
Mall	234a	91	80	Alcohol	Head	T	50	C	G
Mall	172	260	80	Alcohol	.....	T	100	C	G

COLLECTION	NUMBER OF EMBRYO		CROWN RUMP LENGTH OF THE EMBRYO IN MILLIMETERS	IN WHAT MEDIUM MEASURED	REMARKS	DIRECTION OF SECTION T=TRANSVERSE S=SAGITTAL C=CORONAL	THICKNESS OF SECTIONS IN $\mu$	PRINCIPAL STAIN H=HEMATOXYLIN Q=CARMINE	CONDITION OF TISSUE E=EXCELLENT F=FAIR G=GOOD P=POOR
	NUMBER OF SLIDES IN THE SERIES								
Huber	25	85	85	Formalin	Pelvis	S	15	H	F
Mall	170	70	80	Alcohol	Brain	T	100	C	F
Huber	26	70	90	Formalin	Pelvis	S	15	H	G
Huber	20	125	95	Müller's	Incomplete	S	10	H	G
Mall	146	108	95	Formalin	Brain	T	100	C	F
Huber	27	120	95	Alcohol	Pelvis	S	15	H	F
Huber	28	118	100	Formalin	Pelvis	S	15	H	G
Mall	219	48	115	Fresh	Brain	S	$\left\{ \begin{array}{l} 50 \\ \text{and} \\ 100 \end{array} \right\}$	C	F
Brödel	2		120	.....	Injected	T	..	..	G
Mall	120	...	125	Alcohol	Brain	T	100	C	F
Mall	48	191	130	Alcohol	Abdomen	T	100	C	G
Huber	29	146	135	Formalin	Pelvis	S	$\left\{ \begin{array}{l} 15 \\ \text{and} \\ 20 \end{array} \right\}$	H	G
Huber	30	320	145	Formalin	Pelvis	S	10	H	G
Jackson	54	427	150	Formalin	Head	C	75	H	F
Jackson	54	...	150	Formalin	Trunk	T	100	H	F
Mall	220	24	150	Formalin	Brain	T	100	C	P
Huber	31	530	180	Formalin	Pelvis	S	20	H	G
Huber	34	180	180	Formalin	Tissues	..	..	..	G
Minot	727	5	195	Alcohol	Penis	T	10	C	G
Huber	42	...	240	Formalin	Tissues	..	..	..	....



# A CAST OF THE VENTRICLES OF THE HUMAN BRAIN

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## TWO FIGURES

It is the purpose of this paper to describe a method employed in making a Wood's metal cast of the ventricles of the human brain for the use of this laboratory, and to record some results of a comparison of casts made from several different brains.

The casts consist of the lateral ventricles joined by the foramina of Monro to the third ventricle, which is connected with the fourth ventricle by the aqueduct of Sylvius.

A profile view of the cast, fig. 1, shows several impressions and recesses. On the floor of the anterior horn and body of the lateral ventricle may be seen the impression of the caudate nucleus, the groove for the vein of the corpus striatum and the tenia semicircularis, and the impression of the optic thalamus. On the outer side of the body of the lateral ventricle, and extending along the anterior edge of the outer surface of the trigone, is a series of shallow depressions formed by the radiations of the transverse fibres of the corpus callosum, and by the tapetum. On the roof of the inferior horn may be seen, at its extremity, the depression made by the amygdaloid tubercle. The third ventricle shows anteriorly a notch for the anterior commissure, inferiorly the optic recess and the infundibular recess, and posteriorly a notch for the posterior commissure and the suprapineal and pineal recesses. Seen from above, fig. 2, the cast shows the cross striations on the roofs of the bodies of the lateral ventricles.

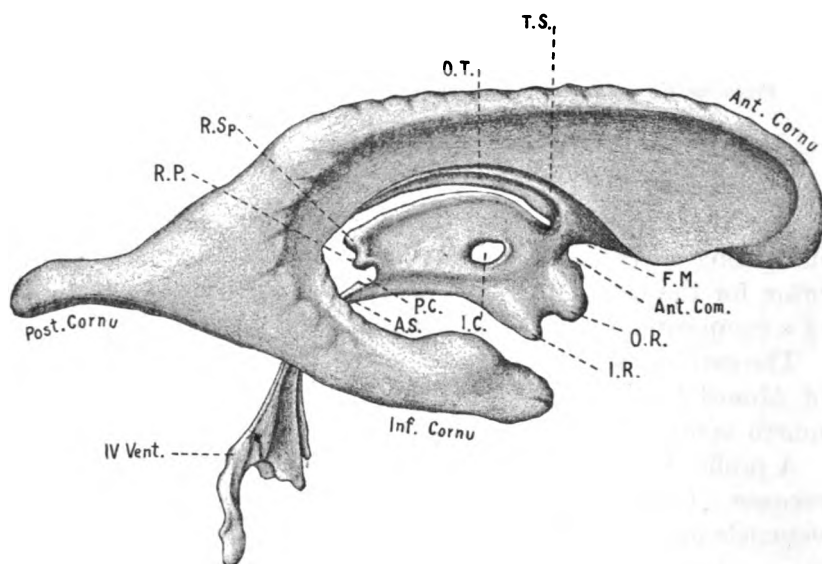
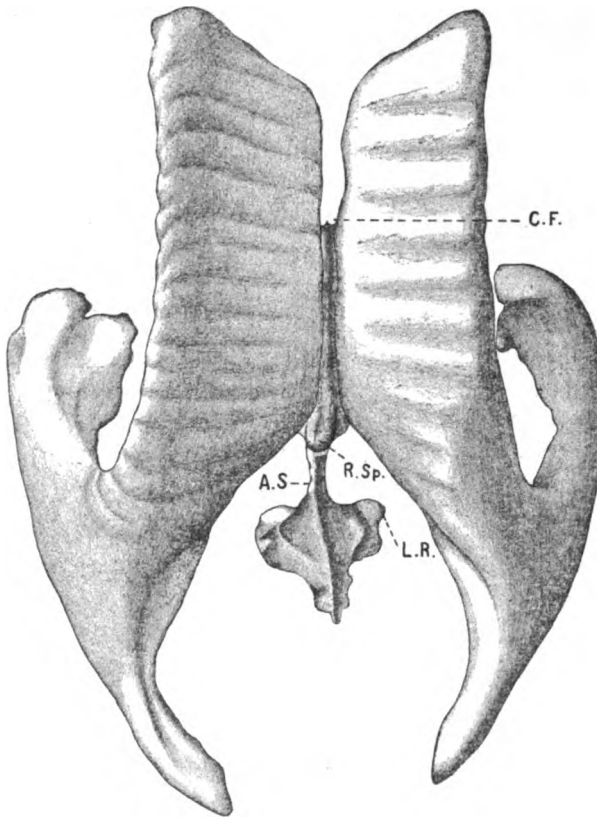


FIG. 1. Profile view of a cast of the ventricles of the brain. Drawn from a photograph corrected to conform with the average. **R. Sp.**, Suprapineal recess; **R. P.**, Pineal recess; **A. S.**, Aqueduct of Sylvius; **I. C.**, Intermediate commissure; **F. M.**, Foramen of Monro; **O. R.**, optic recess; **I. R.**, Infundibular recess; **T. S.**, Tenia semicircularis; **O. T.**, Optic thalamus.



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**FIG. 2.** View from above of a cast of the ventricles of the brain. **R. Sp.**, Supra-pineal recess; **A. S.**, Aqueduct of Sylvius; **C. F.**, Columns of fornix; **L. R.**, Lateral recess.

The method of making the cast was the following: A brain of average size, hardened in formalin, was chosen, and placed in a basin of warm water, where it was allowed to remain for several minutes or until it had become warmed throughout. This procedure was adopted to prevent the premature hardening of the molten metal before it had penetrated the recesses and smaller cavities of the ventricles. Then the brain was removed to a cutting board and cut with a brain knife into four slices by coronal sections through the tips of the anterior horns, the middle of the bodies of the lateral ventricles and the middle of the third ventricle, and the middle of the trigones and anterior end of the fourth ventricle. Parts of the occipital and temporal lobes were cut away to expose the tips of the posterior and inferior horns. The slices were then carefully laid on the board, and with a pair of fine-tipped forceps the chorioid plexuses were removed from the cavities of the ventricles, and the walls of each cavity were thoroughly dried with absorbent cotton. Then the slices were superimposed in their proper order with the brain resting on the occipital poles. The molten Wood's metal was poured slowly from a ladle into the anterior tips of the cavities. When the brain-tissue had been carefully cut away from the cast, irregularities in the metal were smoothed off with a hot iron.

By this method six casts from different brains were made; and from five of these measurements were taken with calipers, the results and averages being recorded in the accompanying table. The dimensions of each part of the cast are in every case the greatest. The casts differ widely among themselves in size and shape; and the two sides of the same cast show wide variations. It will be noticed that the average dimensions of the anterior horn and body of the left lateral ventricle exceed those of the right side. The dimensions of the posterior horns were measured close to the trigones, where they are greatest; and at this point they are greater for the right side than for the left. But four out of the five casts show the left posterior horn to be more extensive than the right, which is in accordance with previous

TABLE 1

*Recording Measurements in Millimeters of the Ventricles of the Brain. D, depth; W, width; L, length*

												AVERAGE	
		L	R	L	R	L	R	L	R	L	R	L	R
Ant. Horn	D	6.0	6.0	11.5	7.5	11.0	10.0	6.5	6.5	5.5	6.0	8.1	7.2
	W	15.5	15.0	22.0	17.5	27.0	17.5	19.0	19.0	21.0	17.0	20.3	17.2
Body .....	D	7.5	6.5	8.5	7.5	13.0	14.0	5.5	3.0	3.5	4.5	7.6	7.1
	W	14.0	12.0	22.0	20.0	24.0	25.5	17.0	17.0	19.0	18.0	19.2	18.5
Post. Horn	D	10.0	8.0	11.0	12.0	10.5	10.0	7.0	10.0	12.5	14.0	10.2	10.8
	W	6.0	5.5	6.5	11.5	14.0	16.0	4.5	7.0	8.0	9.5	7.8	9.9
Inf. Horn ...	D	3.0	5.0	5.0	3.0	4.5	5.0	3.5	3.5	5.0	1.5	4.2	3.6
	W	15.0	18.5	19.0	17.5	*5.5	21.0	14.0	16.0	20.0	16.0	17.0	17.0
	L	25.0		28.0		24.5		25.0		27.0		25.9	
3rd Vent ....	D	16.0		16.0		8.0		16.0		12.0		13.6	
	W	3.5		11.0		12.0		5.0		6.5		7.6	
Aqueduct ...	L	19.0		19.5				17.5		17.5		18.4	
4th Vent ....	D	8.0		6.0				8.5				7.5	
	W	13.5		18.0				17.0				16.2	

\* Not averaged.



observations.<sup>1</sup> In two or three of the brains, the cavities of the right posterior horns were so extremely narrow as to prevent the entrance of the metal in casting; and the casts, therefore, show a blunt posterior horn on the right side, which was the only portion available for measurement. Measurements of the inferior horns show that in two cases the right side exceeds the left, and in two cases the left side exceeds the right. The fifth cast was imperfect, and the measurements obtained from it are not averaged.

<sup>1</sup> Testuté Traite: d'anatomie humaine, vol. ii, p. 706.

# THE CARDIAC GLANDS OF THE MAMMALIAN STOMACH

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In a paper published in 1902 I recorded the results of a study of the so-called cardiac glands in ten different mammals, including representatives of the marsupials, rodents, ungulates, insectivores and primates. The conclusions reached in this article were as follows:

1. The cardiac glands are mucous glands. This conclusion is based on the fact that in every case examined I succeeded in staining the secretory contents of the cells of these glands with muchaematein and mucicarmine. Furthermore, the gradual transition in type of the cells between the foveolae and the bottoms of the glands indicates a close relation between the cells of the surface epithelium and those of the glands which would lead one to suspect the mucous properties of the latter even if the confirmatory stains could not be obtained.

2. The cells of the cardiac glands are fundamentally different from the chief cells of the body of the fundus gland.

3. The cardiac gland cells are closely related to the mucous chief cell of the neck of the fundus gland, and to the pyloric gland cell. This conclusion is based on the fact that all three possess a secretory content which stains strongly in mucicarmine and muchaematein, and that each of the three types is connected with the surface epithelium by transitional cells without an abrupt change at any point, and that each type passes imperceptibly into the other at the margins of contact between successive zones.

4. The cardiac glands are decadent or retrogressive structures derived from fundus glands by the disappearance of the most

highly differentiated elements of the latter, namely, of the chief cells of the body and the parietal cells.

In the same paper also the facts of structure and distribution are reviewed and interpreted in favor of the hypothesis first suggested by Oppel (1898) that the cardiac glands represent stages in an advancing process which ultimately results in the complete suppression of areas of gastric glands and their replacement by a mucous membrane of the oesophageal type with a stratified epithelium.

In two recent articles dealing with the cardiac glands Haane (1905, 1 and 2) has arrived at conclusions which are the exact opposite of mine, and which may be summed up by saying that he regards these structures as non-muciparous glands differing from both the fundus glands and pyloric glands, and having a specific function, namely, the secretion of an amylolytic enzyme. Haane's first paper was written without knowledge of my observations, and in the second paper he calls attention to the conflicting results obtained by me and reaffirms his confidence in his own conclusions.

The characters upon which Haane lays greatest stress in defining the cardiac glands are derived chiefly from his study of these structures in the pig, in which, as is well known, they occupy a much larger area of the mucous membrane than in any other of the domestic mammals. These characters are as follows: The cardiac glands are tortuous, tubular or tubulo-alveolar glands, which lie without special grouping in the propria mucosae, and whose cells stain with eosin and other acid dyes; they do not react to mucus stains. From the pyloric glands, with which they could be most easily confused, they are clearly different. The latter form groups in the gastric mucous membrane, exhibit the mucus reaction, and have a different form and course.

Applying these criteria to the glands found in the usual location of the cardiac glands he is led to rather surprising results. The glands which are found in the horse along the fold which separates the glandular and non-glandular portions of the stomach, which were previously interpreted by Ellenberger and Edelmann as cardiac glands, Haane says are not cardiac glands but pyloric

glands, because they stain with mucus dyes and agree in their properties with pyloric glands. In the ruminants he found that the glands occupying the strip where the psalterium joins the glandular stomach were different in the three species examined, resembling pyloric glands in the ox and cardiac glands in the sheep and goat. In the ox these glands stained with mucicarmin and were only slightly stained by eosin, while in the sheep and goat the converse was true.

Finally, Haane made extracts of the mucous membrane of the cardiac region of the pig and tested them for various enzymes, obtaining negative results with respect to pepsin, rennin, lipase, lactase and invertase, but strongly positive results for amylolytic enzyme. He adds, however, that extracts of the fundus and pyloric mucous membrane and of the glandular membrane of the oesophagus gave equally strong evidence of the presence of amylolytic ferment. That of the fundus mucous membrane was even stronger in this respect than the extract of the cardiac region.

More recently the cardiac glands have been studied by two other workers in Ellenberger's laboratory, namely, by Fröhlich (1907) and Mönnig (1909). Fröhlich confirmed the existence of cardiac glands in all domestic mammals, but noted that there were great differences in structural detail in different species, as well as in the chemical properties of the constituent cells. His conclusions as to the phylogeny of the cardiac glands are in accord with those previously reached by Oppel and myself, inasmuch as he regards the cardiac glands as phylogenetic simplifications of the fundus glands. Notwithstanding the fact that he was unable to stain the cardiac glands of the pig with mucus stains, he yet came to the conclusion that they were probably muciparous, assuming that the secretion was not sufficiently elaborated in the cell to give the mucin-reaction with dyes. As confirmatory evidence, he brought out the fact that the contents of the cells of the cardiac glands of the pig, unlike the serous cells, became turbid when treated with acetic acid, although less so than the cells of the surface epithelium and of the pyloric glands. He also studied the transition between the cardiac gland zone and the pyloric gland zone on the one hand and that between the fun-

dus and cardia on the other hand, and established the fact, to which I have elsewhere referred, of the existence of glands intermediate in type between those of the adjacent zones in question.

Mönnig (1909) studied the cardiac glands of the pig only, coming to conclusions which are in general accord with those of Haane, inasmuch as his experiments with various methods of staining mucin gave uniformly negative results in the cardiac glands of the pig. Furthermore, by means of the Altmann method for the demonstration of cell granulations, he succeeded in demonstrating in the cells of the cardiac glands of the pig, granules which, in their refractive power and their tinctorial reactions, resembled the granules of the parotid gland, and which, he assumed, therefore, to be zymogen granules.

It will be observed from the foregoing résumé of the literature on this topic, that there are two views concerning the nature of the cardiac glands which are directly opposed to one another. According to Oppel (1898) and Bensley, they are decadent or regressive structures, derived from fundus glands, and constituting a stage in an advancing process which will result ultimately in the complete suppression of the glands in the area which they occupy. According to this view they would be physiologically of little importance, although Bensley regards them as muciparous. On the other hand, Haane and Mönnig regard them as progressive elements which have arisen in mammals as a response to a new functional demand. According to this view they are important secretory elements yielding a digestive enzyme.

Before proceeding to a discussion of these opposed views, it may be well to point out that there are no cardinal points of difference between the several authors cited, as regards the structure and distribution of the cardiac glands in the different mammals, if we except the interpretation of the cardiac glands of the horse and ox as pyloric glands by Haane. The differences of opinion have to do rather with questions concerning the staining properties of the secretion of the cells, and with the interpretation of the results with reference to the origin and function of the glands.

In view of the fact that both Haane and Mönnig obtained uniformly negative results in the cardiac glands of the pig, where I had previously reported positive results with mucus stains, it seemed important to re-investigate these structures and to determine with greater precision the conditions under which these positive results could be obtained. Accordingly, I have studied the reactions which the secretion in these cells give with several mucus stains after fixation in a number of different fluids.

The fixing solutions are as follows:

- (1) Alcohol.
- (2) Saturated solution of mercuric chloride in alcohol; 2.5 per cent solution of potassium bichromate in water. Mix equal volumes.
- (3) Zenker's fluid.
- (4) Formaline, 1 part; Zenker's solution without acetic acid, 9 parts.
- (5) 2 per cent osmic acid, 4 cc.; 2.5 per cent pot. bichromate, 16 cc.; Glacial acetic acid, 2 drops.
- (6) Altmann's osmic bichromate solution.
- (7) Saturated solution of mercuric chloride in water.

The following staining solutions were employed:

- (1) Strong muchaematein:
 

Aluminum chloride.....	g. 0.5
Haematein.....	g. 1.0
70 per cent alcohol.....	cc. 100
- (2) Mayer's muchaematein.
- (3) Mayer's mucicarmine (stock solution).
- (4) Weak mucicarmine-solution no. 3, diluted with ten times its volume of tap water.
- (5) Toluidene blue.

The muchaematein solutions were ripened for one week before using, and then brought to the optimum reaction for staining mucus, by the careful addition of nitric acid, in the manner recommended by Mayer (1897).

The time of staining did not exceed five minutes, except where the result was negative, in which case the staining was prolonged for several hours to confirm the negative results. After the staining was completed, except in the case of the toluidene blue, the sections were washed in 70 per cent alcohol (washing in water spoils the result), dehydrated in absolute alcohol, cleared in benzole, and mounted in balsam. The toluidene blue sections were

examined in water to determine the distribution of metachromatic staining.

For purposes of comparison, sections of the fundus and of the pylorus were stained side by side with those of the cardiac region. The results of these experiments are recorded in table 1. On account of the fact that the mucous cells of the neck of the fundus gland stained differently in some cases from those of the body of the gland, the results, as far as these two groups are concerned, are recorded separately. In the table, a positive sign indicates that all the cells of the gland gave a positive reaction; a negative sign that all were negative; both positive and negative signs that some cells reacted positively, some negatively in the same preparation. As some differences were noted between the glands from different animals, according to the functional state of the cells, two additional series of observations of the cardiac glands are added. A positive sign after toluidin blue indicates metachromatic staining of the secretion of the cells in question.

Before proceeding to a consideration of these results it is well that we recognize the limitations of the so-called mucin stains as a means of determining whether a cell does or does not secrete mucin. These were well known to Paul Mayer, to whom, more than to any other, we owe the progress that has been made in this branch of technique. In his article entitled "Ueber Schleimfärbung" (1897), he makes the following statement: "Oben habe ich bereits angedeutet, dass die Schleime sich gegen einen und denselben Farbstoff sehr verschieden verhalten, indem ich bei Besprechung der Lösungen von Hämatoxylin oder Hämatein sagte: in der Regel färbt sich der Schleim oder in der Regel färbt er sich nicht. . . . Man könnte da eine förmliche Reihe aufstellen, die mit solchem Schleime zu beginnen hätte, dessen Färbung kaum zu verhindern ist, und mit solchem enden würde der sich kaum noch färben lässt." Such a series can be constructed even within the limits of a group of homologous glands as I have shown in the case of the glands of Brunner (1903). Here I found that the glands of Brunner of the squirrel would stain blue even in an alum haematein solution, while those of most other mammals required for this purpose the stronger muchhaematein solution, and those of the sheep stained but slightly even in that.

TABLE 1

*Showing results of experiments in staining the gastric glands of the pig in several mucus stains, after various fixations.*

STAINS	FIXING SOLUTIONS						
	ALCOHOL BICHR. SUBLIM.	ALCOHOL	ZENKER	FORMAL. ZENKER	AQU. SUBLIM.	ACETIC OSMIC BICHR.	ALTMANN
SERIES 1							
<i>Cardiac glands</i>							
Strong muchaematein	+ -	+	+	-	+	+	-
Weak muchaematein	+ -	+	+	-	-	+	-
Strong mucicarmine	+ -	+	+	-	+	+	-
Weak mucicarmine	+ -	-	-	-	-	+	-
Toluidin blue	-	-	-	-	-	-	-
<i>Neck-chief cells of fundus glands</i>							
Strong muchaematein	+	+	+	+	+	+	+
Weak muchaematein	+	+	-	+	+	+	+
Strong mucicarmine	+	+	-	+	+	+	+
Weak mucicarmine	+ -	+	-	-	+	-	-
Toluidin blue	+ -	-	-	-	-	-	-
<i>Mucous cells of body of fundus gland</i>							
Strong muchaematein	+	+	+	+	+	+	+
Weak muchaematein	+	+	-	+	+	+	+
Strong mucicarmine	+	+	-	+	+	+	+
Weak mucicarmine	+ -	-	-	-	+	-	-
Toluidin blue	+ -	-	-	-	-	-	-
<i>Pyloric glands</i>							
Strong muchaematein	+	+	+	+	+	+	+
Weak muchaematein	+	+	+	+	+	+	+
Strong mucicarmine	+	+	+	+	+	+	+
Weak mucicarmine	+ -	+ -	+ -	+	+	+	-
Toluidin blue	+ -	+ -	+ -	+	+ -	+	-
SERIES 2 AND 3							
<i>Cardiac Glands</i>							
Strong muchaematein	+	+	+	+	+	+	+
Weak muchaematein	+	+	+	-	-	+	-
Strong mucicarmine	+	+	+	+	+	+	+
Weak mucicarmine	+	-	+ -	-	-	-	-
Toluidin blue	-	-	+ -	-	-	-	-



The question is still further complicated by the fact that these solutions may occasionally give positive results in glands where a chemical examination of the secretion reveals not the slightest trace of mucin. Such a case Mayer found in the submaxillary glands of the hedgehog.

In view of these facts, it may be said that the results obtained from the use of the so-called mucus stains are suggestive or confirmatory rather than demonstrative, and that they should only be interpreted in connection with evidence derived from the chemical examination of the secretion, or, when this is impossible, from the consideration of the information that may be elicited by a study of the morphological relationship of the cells in question to epithelia of known function.

By reference to table 1 it will be seen that, of thirty-five combinations of fixing and staining, in series 1, twelve gave positive results for mucus in all the cells of the cardiac glands, four gave positive results in some cells, negative in others, while nineteen were negative. The strongest stains were obtained after fixation in alcohol, but tissues fixed in Zenker's fluid or in the acetic-osmic-bichromate mixture also gave strong stains. Only two of the fixations gave negative results in all the stains, namely, formaline-Zenker and Altmann's fluid. In series 2 and 3, however, in which the experiments were carried out in the same way, cardiac glands fixed in these fluids gave positive results with the strong muchae-matein and strong mucicarmine, while tissues from the same animals fixed in alcohol-bichromate-sublimate gave positive results instead of partial as in series 1. This was apparently due to the fact that the cells were in a different physiological state from those of series 1.

In the neck-chief cells of the fundus glands the results were positive in twenty-one, partial in two, and negative in twelve. In the mucous cells of the body of the fundus gland they were positive in twenty, partial in two, and negative in thirteen. In the pyloric glands they were positive in twenty-six, partial in seven, and negative in two. These results are expressed in table 2:

TABLE 2

*Summary of results on staining the gastric glands of the pig with mucus stains*

## SERIES 1

	POSITIVE	PARTIAL	NEGATIVE
Cardiac glands.....	12	4	19
Neck-chief cells of fundus glands.....	21	2	12
Mucous cells of body fundus glands.....	20	2	13
Pyloric glands.....	26	7	2

## SERIES 2 AND 3

Cardiac glands.....	19	2	14
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The results tabulated as partial are those in which the secretion in some of the cells stained while that in others remained unstained. This result is obviously to be interpreted as due to the phase of chemical elaboration which the secretion in the particular cell had reached, and as indicating that this secretion reacted positively with greater readiness in certain phases of its elaboration than in others.

Conservatively interpreted, these results indicate that, in so far as staining reactions are concerned, the probability that the several types of cells considered are engaged in the production of a form of mucus, is 94 per cent for the pyloric glands, 63 per cent for the chief cells of the neck of the fundus gland, and 60 per cent for the cardiac glands (series 2 and 3). The positive results in each case by no means prove that the cells are mucous cells; they only indicate that that is a reasonable supposition.

We are obliged, therefore, in order to get further evidence as to the function of these glands, to fall back on other sorts of evidence. Of course, the method that at once suggests itself is the chemical examination of the mucous membrane or of the secretion from a permanent fistula of this region. Here, however, such methods can help us but little, for, whether we make extracts of the mucosa, or examine the secretion, we are sure to find mucin, derived from the surface epithelium. The discovery of a specific enzyme in the cardiac glands would undoubtedly favor the view

that the glands were not mucous glands, but as yet such evidence is not forthcoming. It is true that Haane (1905) demonstrated the presence of an amylolytic enzyme in the extracts of this region, but he also demonstrated that it was not specific for this region, when he found that extracts of the fundus were even stronger in this respect than those of the cardiac region. If we accept Haane's conclusion that the cardiac glands are specific elements different from anything in fundus glands, then we must assume that his amylolytic ferment did not come from the glands, but from elements which he admits the fundus and cardiac glands to have in common, namely, the surface and foveolar epithelium. If we reject Haane's hypothesis, then we must assume that the cardiac glands have a common function with those elements in the fundus glands which remain after the exclusion of the types of cells which are not represented in the cardiac glands; in short that they share the function of the accessory cells of the fundus glands, which for many reasons are believed to be mucous cells.

There are, however, other very clear indications that the conclusion drawn from the staining reactions that the glands are mucous glands is correct.

Of first importance in this connection is the close relation that exists between the cells of the cardiac glands and those of the surface epithelium of the stomach. If one examines the transition between these two types he will find that there is no point where an abrupt change in character of the epithelium takes place, such as is common in other glands. On the contrary the transition is a very gradual one, and it is easy to find, in the upper territory of the gland, cells which are precisely intermediate in type between those of the bottom of the gland and those of the surface. The change from one type to another is accomplished by gradations which are almost imperceptible from cell to cell, although the extremes are very different. It is of course, possible that the cells of these intermediate types are engaged in a two-fold function, sharing in this way the mucigenous function of the foveolar epithelium and the unknown function of the gland cells, and that, proceeding in one direction, the latter function becomes increased to the ultimate exclusion of the former. But, when we consider

the staining reactions with mucus stains, this does not seem probable, and it does not agree with our experience in other glands. For example, there is an abrupt change from neck-chief cells to body-chief cells and no intermediate types. The same is true of the transition of the glands of Brunner where they open into intestinal crypts, and of the relations of duct and acinus in all the salivary, buccal and oesophageal glands. In the stomach only do we find this gradual transition from one type of cell into another, and it appears to me that the simplest explanation is that the differences are really less than they seem and that the two types so related morphologically are also physiologically related and are concerned with the production of a similar secretion.

In this connection it may be remarked that, although accurate information on the subject of the composition of the slime secreted by the gastric epithelium is still lacking, the work of Cremer (1895) shows that it owes its physical properties to the presence of a true mucin.

Another argument in favor of the interpretation of the cardiac glands as mucous glands is derived from the consideration of the nature of the glands which occupy the intermediary zones between the cardiac and fundus regions on the one hand and between the cardiac and pyloric regions on the other. These intermediate zones have been carefully studied by Fröhlich (1907), who showed that, although pyloric and cardiac glands are very different in appearance, when compared at points some distance from one another, at the margin of contact of the two zones, these two types are connected by a series of intermediate types constituting a perfect transition between the extremes. Similarly, on approaching the fundus region the cardiac gland cells become more and more like the mucous cells of the fundus glands. In consideration of these facts the evidence of relationship between the pyloric glands and the cardiac glands indicates that they have a common function which, however, they may exhibit in different degrees of intensity.

These transitions indicate that the surface and foveolar epithelium and the cells of the cardiac and pyloric glands and the mucous cells of the neck of the fundus glands constitute a group of

closely related functional elements which differ it is true, *inter se*, but which, nevertheless, engage in a similar function. As we know the foveolar epithelium and the pyloric glands to be mucous glands, it is probable that all belonging to this group are also mucous glands. Whether, as is probable, they secrete other substances as well, we can not say at present.

We may now proceed to a discussion of the facts which have been advanced by Haane and Mönnig, in support of their view that the cardiac glands in the pig are serous elements having a zymogenic function. Haane bases this view on the negative results obtained by him in the cardiac glands of the pig with mucus stains, on the greater affinity for eosin shown by these glands as compared with the cardiac glands, and on his success in extracting a diastase from the mucosa. To these Mönnig adds the observation that the cells of the cardiac glands of the pig contain a secretion antecedent in the form of refractive granules, which, when fixed and stained by the Altmann method, resemble the secretion granulations of the parotid gland. These, he says, must be interpreted as zymogen granules.

I have already considered the negative results of Haane and Mönnig, and have shown how positive results may be obtained in staining the cardiac glands of the pig with mucous stains. There still remains to be considered the reaction of the cells to eosin, and the granules which Mönnig interpreted as zymogen granules. With regard to the former, it is apparent that Haane has made no attempt to analyze the reasons of this affinity for eosin, for he does not tell us whether it is the secretion or the cytoplasm or both which stains with eosin. Fröhlich, on the other hand, in his descriptions of the cardiac glands, is careful to specify what portion of the cell stains with eosin. From his descriptions, with which my own observations wholly agree, it appears that the portion of the cell which stains is not the mass of secretion, but, on the contrary, the protoplasm of the cell. This protoplasm stains with eosin or other acid dyes as does all protoplasm which has been fixed in acid solutions or in mercuric chloride, unless there happens to be a specific basophile substance present, as is the case in the chief cells of the fundus glands. When a gland

cell stains strongly in eosin, therefore, it indicates that there is little chromidial substance present, and that the proportion of cytoplasm as compared with secretion-antecedent is relatively large. A mucous cell, thus, will stain strongly in eosin or otherwise, according to the stage of secretion in which it happens to be when fixed, and, by the simple expedient of stimulating the gland, cells with a strong affinity for eosin may be produced in any mucous gland. The reaction with eosin is thus by no means an argument in favor of the interpretation of these cells as serous cells. On the contrary, the absence of the basophile chromidial substance, which this reaction indicates, speaks strongly against this conclusion, for cells which we know to be serous, as for example, the parotid gland, the chief cells of the gastric gland and the pancreatic cells, have been shown to contain this substance and to increase it after stimulation.

Mönnig's observation that the cells of the cardiac glands of the pig contain in their distal segments granules which, like those of the parotid gland, may be demonstrated by the well-known Altmann method, I can fully confirm. I cannot, however, accept his conclusion that these are zymogen granules. In this connection Mönnig remarks that the richness of these cells in granules varies with their functional condition, but that he has not investigated these changes. This hiatus I am now able to fill. I have found that the number of the granules of this sort varies, as stated by Mönnig, with the condition of the cell. In some glands the entire secretory content of the cells of the cardiac glands is in the form of fuchsinophile granules, which are best demonstrated by the Altmann method. In glands from another animal, however, the cells may contain more reserve secretion-antecedent than in the case just cited, but only a small portion is in the form of fuchsinophile granules. The rest consists of granules of very low refractive index which do not stain in the Altmann method. The latter granules, however, stain with strong muchaematein and strong mucicarmine even after fixation in Altmann's solutions, and as indicated in table 1, series 2 and 3, when fixed in alcohol, Zenker, or acetic-osmic-bichromate, they stain in the weak muchaematein as well. The fuchsinophile

granules are, therefore, not the proximal antecedent of the secretion, but are transformed before secretion into another antecedent which, both in the fresh cell and in fixed tissue, has the appearance and reactions of mucus. The presence in mucous cells of a remote antecedent in the form of granules of higher refractive power than the mucigen granules, which stain with acid fuchsin but not with mucus stains, has been demonstrated by Krause (1885) in the retrolingual gland of the hedgehog, and by myself (1903) in the pyloric glands of *Cavia* and *Plethodon*, and in the epithelial cells of the stomach of the young pig. I have also shown that under certain conditions of fixation the fuchsinophile granules stain with mucin stains in the cardiac glands of the pig (table 1, series 1).

We may conclude, therefore, that the probability of the correctness of the conclusion that the cardiac glands are mucous glands, which has been drawn from their staining reactions and from a consideration of their relationship to epithelium of known secretory function, has not been diminished by the consideration of the facts brought out by Haane and Mönnig, and that these facts are consistent with the known facts concerning the mucous cells, and the changes they show in different phases of their secretory history.

To the question of the phylogenetic source of the cardiac glands, Haane devotes no attention in his articles, and Mönnig merely states that he regards the occurrence of a graded transition between the cardiac glands and the fundus glands on the one hand, and between the former and the pyloric glands on the other hand, as no indication that the cardiac glands have been developed from either. He remarks that one might with equal justice claim that the pyloric glands have been developed from cardiac glands. It is apparent that he has not attached sufficient weight to the facts concerning the distribution of the various sorts of glands in those simple mammalian stomachs which, we have good reason to believe, represent the primitive condition. Indeed, not only is there great uniformity in this respect in simple mammalian stomachs, but the latter agree in their main characteristics with those of lower vertebrates down to the fishes. In all, there

is a well-defined fundus gland area occupying the proximal end of the stomach, and an equally well-defined pyloric gland area occupying the distal or pyloric end. Moreover, in *Batrachia*, *Reptilia* and *Mammalia*, the relations of the two types of glands to one another, as regards their cellular constituents, is constant, inasmuch, as, in these three orders, the pyloric glands are composed of cells similar to cells which occupy the neck of the fundus gland. Thus the fundus glands and the pyloric glands are structures which have a long phylogenetic history and a considerable morphologic fixity of type. The cardiac glands on the other hand appear for the first time in *Mammalia*, and occupy a territory which in more primitive stomachs is occupied by fundus glands. If we assume, as did Haane and Mönnig, that they are new structures developed for a special purpose in Mammals, and having no relation to the fundus glands, we must explain why the fundus glands which originally occupied this area have disappeared and what the functional need is that these structures serve. No evidence of value is advanced by Haane and Mönnig on either of these topics, for Haane has shown that, as far as the formation of his amylolytic enzyme is concerned, this function is even now better performed by the fundus glands than by the cardiac glands which have replaced them.

The alternative hypothesis is that in the course of evolution the fundus glands which occupied this region have been brought to a successively less and less perfect development in succeeding generations and that the cardiac glands represent one result of this regressive process. If this be true, it is probable that they are vestigial physiologically as they are morphologically.

It is not the purpose of this paper to review at length the arguments in favor of this hypothesis, in view of the fact that I have already dealt with this question fully elsewhere (1902). I may point out, however, that Fröhlich (1907), who is the only one of the authors quoted who has considered the question fully since the publication of my article, has come to a similar conclusion, and that this conclusion is supported by the histogenetic studies of Kirk and myself, which show that the embryonic cardiac glands of the pig contain parietal cells which later disappear.



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## THE QUESTION OF APPLIED ANATOMY

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The demand for practical teaching in anatomy, and for the practical, has been an insistent one for decades. Unfortunately the term "practical" was often used as opposed to the scientific by those who made these demands. All facts which did not have a very patent application were spoken of as abstract, or theoretical even. That the teaching of the scientific could not be of any practical utility, and that the inclusion of the practical relations sacrificed the scientific, were long accepted as truisms by many professed devotees of anatomy. The scientific and the practical were supposed to be mutually exclusive and antagonistic, and necessarily so. That in conjunction with other things such misconceptions did not hasten the advent of scientific anatomy, in this country, is better known to many anatomists than to myself. It is not my purpose to consider here what the effect of this so-called practical teaching and of such views has been upon the teaching of anatomy and upon the organization of departments of anatomy. Whatever their effect it is clear to every one that rapid progress is now being made by a movement of truly national scope, and that the names of the few who initiated this movement will ever be held in grateful remembrance. Perhaps no one will gainsay that the training of the future practitioner of medicine shall be a practical one. Every one recognizes that the prevention and alleviation of suffering are the first duty of the physician. Hence, his interest in those facts which have found direct application is a paramount one. But his attention must not be directed solely and his interests confined exclusively to them. The needs of the hour of the practitioner must not become the alpha and omega of student life, for the purely theoretical and scientific of to-day may perchance be, and often have become, the intensely practical and utilitarian of to-morrow. In spite of the strictures passed upon a great national institution a

few years ago for the accumulation of what were termed "mere facts." I take it that it lies within the power of no one, be he seer or prophet, to judge or to predict the value of an isolated scientific fact. Of books and men many may be insignificant, but not of facts. These are manifestly such only because of ignorance. As Sherrington well said: "In nurturing science, I would urge that the community cultivates more than mere utility; and even in regard to mere utility, as the fields of knowledge fall ripe under the ceaseless husbandry of the world's thought, those who would join in the great reaping, and not only glean where others reaped before them, must cultivate for themselves."

That it is possible to practice medicine without contributing to the development and advancement of the medical sciences, is not denied. This has received abundant demonstration. Yet who will maintain that it can be done as efficiently? Hence, the future licentiate must be trained for growth. He should have a broader horizon and a more fundamental training than can be obtained by mere familiarity with isolated well-established facts and well-tried routine procedures of the day. "It is necessary that the student go forth from his school equipped not only with the present applications of science to disease, but so possessed of the root principles of the sciences adjunct to medicine that he may grasp and intelligently use the further development of scientific medicine after he is weaned from his instruction and the school. That is the way to obtain enlightened progress in professional practice. What truer safeguard can a man have, alone it may be, and isolated from the centuries of knowledge, what truer safeguard can he have against all the pseudo-scientific quackeries of the day, than some real knowledge of the principles of the sciences along whose lines the discoveries of medicine must develop?" (Sherrington) Principles rather than details, then, must guide the practitioner of the future, and biological laws rather than facts must point out the line of attack upon the many new problems with which he will come face to face. The memorizing of Gray, or quiz-compend methods, cannot help him, though they enable him to pass state-board examinations with the best of marks, or take the highest honors. And regarding honors, was it not Stevenson who said, "Though here and there a Lord Macaulay may escape from school honors with all his wits about him, most boys pay so dearly for their medals that they never afterward have a shot in their locker and begin the world bankrupt?"

The old demand for the practical, however, persists although the student who has become interested in a subject, be it ever so far removed from utilitarian ends, seeks such props no more than the teacher who

really lives in the field in which he works. This is as true, perhaps truer, of human anatomy than of any other subject. Hence, the necessity for teaching applied anatomy must lie in the requirements of the future practitioner, or the exigencies of the situation, rather than in the necessity for arousing interest in anatomy itself. I have elsewhere expressed myself to the effect that I fail to see why many of the things usually included in this subject should be taught at all in the required work of the curriculum, and that many of the remaining relations can be discussed partly in connection with the work in systematic anatomy, and mainly in connection with topographical anatomy leaving to the surgeon the things that are his. The question besides is one of time and of organization.

There seems to be no consensus of opinion, however, on what shall constitute applied anatomy. At the present it may mean topographical anatomy, partly normal and partly pathological, regional topographical anatomy, surgical anatomy, medical anatomy, or anatomy as related to the whole field of medicine and surgery including the specialties—sometimes called clinical anatomy, or even operative surgery. It is clear, of course, that all these things save operative surgery would be included in the term “clinical applied anatomy.” Besides these, there are, of course, the relations of anatomy to the fine arts, like sculpture and painting, to hygiene and the related subjects of physical education, to the industries such as the designing of wearing apparel, furniture, seats, chairs, etc. Indeed, shoes and seats are usually made as though body form were wholly undetermined and indeterminable. With the exception, perhaps, of artistic anatomy, the discussion of these matters might be of more real value to the student than much that is now included in so-called applied anatomy. It is not my purpose, however, to suggest that all the above should be included, save in a purely optional course. Indeed, all I am concerned with at present is that there should be some fairly definite understanding as to how much of this work, and which of it, if any, should be included in the required work of the curriculum.

Any one who has examined the books issued on applied anatomy in the last decade or more, either as first or revised editions, must have been impressed with the fact that most of them deal almost exclusively with surgical applied anatomy and with operative surgery. Consequently, they are suited for the use of the surgeon rather than for that of the student of medicine, and, although we have been assured that “the number of excellent works on applied anatomy is large enough to render the exhaustion of an edition of any one a fair presumption of its fitness

to survive," it is likely that an *excellent* book on applied anatomy for the student of medicine is yet to be written. Most of the existing texts are indeed surgical applied anatomies, with a varying amount of operative surgery. By far the best of these books which has come to my attention is the well-known little volume by Sir Frederick Treves, called "Surgical Applied Anatomy." In the introduction to this volume, it is frankly stated, that it "is intended mainly for the use of students preparing for their final examinations in surgery." Unfortunately, meeting the examinational requirements of British students has often been made one of the avowed objects of English textbook writers—may I say it?—since or before the days when Sir Charles Bell, great as he was, wrote on the "relation of arteries to surgical operations, especially for those who dissect and desire to take examinations." While the onus of this may easily be borne by men of such eminence, yet when quiz-compend aims are frankly adopted in writing textbooks, it is time to recall that the requirements of a subject and the standards of medical education, rather than licensure tests should determine the character of books deserving careful consideration, unless, as we do not believe, it still be the object of British medical schools, as it is of some of ours, to train students for tests rather than for life. Moreover, since Treves' "Surgical Applied Anatomy" considers such things as Chopart's, Lisfranc's, Pirigoff's and Symes' amputations, and excisions of the superior and inferior maxilla, and shoulder amputations, it is clear that it should not be used indiscriminately in our schools. If all teachers of applied anatomy, or of anatomy, who use textbooks outlining all manner of major operations in surgery, were men of corresponding standing, we might be content to let the matter rest there. But, naturally, this work often is and probably long will be done by those who are neither experienced surgeons nor anatomists. Hence, while a consideration of the technique of major operations in surgery may be all good and well enough for students and practitioners of surgery, the rank and file of medical students who according to every one should never attempt these things without careful post-graduate hospital training, cannot profit by them.

It is not my intention to write a review of recent books on applied anatomy, but reference to a few may be of interest. As is customary, anatomical facts of importance to the surgeon only are given. Yet the author of one of these very recent volumes hopes that the detail given will be sufficient "to obviate frequent reference to textbooks of systematic anatomy." Some of the clinical and anatomical facts given in this book include the statements that "trigeminal neuralgia is believed

to be due to contraction of the dural pocket, containing the ganglion, which is therefore *crushed*." Pain due to "night startings" is said to be due to "a relaxation, and possibly jerking, of these muscles on sleep supervening, producing a jarring of the bones on one another, the delicate nerve fibrils which exist in such abundance just under the articular cartilage being *crushed*." (The italics are the writer's.) This same text also copies a longitudinal section of the spinal column with the cord *in situ*, in which the latter is represented as large as the bodies of the vertebra. "The mediastinum," we are told, "extends from the sternum to the spine, forming a complete septum between the lungs, and is deflected somewhat toward the left. It is bounded by the diaphragm below, but is open above, while laterally it is bounded by the pleura." The author, evidently being undecided whether the mediastinum is a space or a septum, adopts both conceptions, and then bounds the septum by itself! This book also contains facts so invariable and so essential to the surgeon as that the "nutrient artery of the tibia is the largest in the body," and that "the sacrum is really suspended between the innominate bones by its ligaments." Besides these things, the whole book is carelessly written, quite in contrast to the terse, clear English for which many British textbooks are deservedly known. The student is told, for example, that "When the bladder is much distended the orifice is slightly depressed, while if the rectum be distended it is slightly raised. Normally, it lies behind and slightly below the level of the upper margin of the symphysis pubis." Were it not for the many able and eminent surgeons both here and abroad who repudiate such teaching, one might be tempted to suggest that, while such anatomical facts may suffice for the surgeon and for those preparing to pass British licensure tests, any one worthy of the name anatomist would be unwilling to teach them. It is also difficult to see how a novitiate in practice without first-hand experience gained at the operating table, can profit by such descriptions as the following: "Amputations of the shoulder may be performed by making a racket-shaped incision to include a deltoid flap from the outside of the coracoid down to the lower border of the pectoralis major, then outwards across the limb through the lower portion of the deltoid to the posterior axillary fold, the limb being abducted and rotated outwards. As the first part of this incision is practically that for excision, it enables the condition of the parts about the joint to be examined before proceeding to amputate, in case of doubt. The racket is now completed across the superficial tissues on the inner side of the limb. The deltoid flap, containing the posterior circumflex vessels and circumflex nerve, is now

raised, the capsular muscles, capsule and triceps tendon divided; and the head disarticulated. The triceps, latissimus dorsi, and teres major, are next cut, and the limb being drawn from the side, the axillary vessels may be ligatured, and then along with the nerves, cephalic vein, humeral branch of the acromio-thoracic artery, some fibers of the deltoid and triceps and coraco-brachialis, divided by a transverse incision." Put this into the hands of the recent graduate, with or without a word about the conditions which justify it, and submit your shoulder, if you like, my friend. This same text gives a similar account of Kraske's operation for carcinoma of the rectum, etc., and, since it does not profess to be an operative surgery, it may pertinently be asked of what use such things are save to enable students to pass examinations which they should not pass, and perform operations which they should never perform.

The purpose of another recent book is "to provide the student with a handbook dealing not only with surgical and medical anatomy but with operative surgery." Hence the fact that "two classical operations seldom performed nowadays have been allowed to remain on account of their historical interest" is not to be wondered at. No doubt gynæcologists as well as anatomists will commend the author of these volumes for concluding that "the surgery of the female generative organs has now become so large a subject that it has been thought possible to omit descriptions or gynæcological operations altogether in the present edition." Even with this omission, it is to be doubted whether American medical students or their future patients can be benefited by instruction which considers iridectomies and cataract extraction, even were this instruction to be given in surgery rather than in applied anatomy.

An attempt has also been made by an American surgeon to bring together what he considers of interest to all medical students. The aim is a worthy one, and we hope that ere long a textbook of applied anatomy, not an operative surgery, will be written by some one fully qualified to do it. In this connection it may be recalled that some textbooks of systematic anatomy retain chapters on practical considerations, much after the manner of Gray. The reason given in Gray, however, for including a large amount of surgical anatomy, was that it was hoped to meet the needs of the practitioner in surgery and not the rank and file of medical students. In an American textbook of systematic anatomy, the high character of which has received well-merited recognition, in spite of the great need for revision, practical relations are also discussed to illustrate "the dependence of diagnosis and practice upon anatomical knowledge, to awake interest and to combat the tendency to regard anat-

omy as something to be memorized during student days and to be forgotten when examinations are over," and because it was thought "that it will make it easier for the student to learn his anatomy and for the physician to remember and apply it." Whatever one may think of the wisdom of introducing these things, or their efficiency in accomplishing the ends declared, every one certainly recognizes the worthiness of the aims.

There seems to be practical unanimity regarding the demand that students of medicine shall at least satisfactorily dissect the lateral half of the human body. Although this is an absolutely fixed quantity, yet, as every one knows, it is covered in a period of time varying from two hundred to four hundred hours or more. The amount of time spent by the student varying as a rule inversely with the ability of the student and the standing of the school. It is also true that the amount of time required of the student for this work in some of our best medical schools is no criterion of the amount of time actually spent by him. For as long as well-qualified students only are admitted, high standards of work are set and *maintained*, and sufficient time is reserved in the schedule with or without the introduction of the elective principle, it matters not how much or how little is demanded. As has been well shown, under these conditions, good students do much more than is required of them, while the weaklings who slight their work are easily eliminated by a process of daily supervision and by means of practical instead of memory-test examinations. The other extreme in this matter is represented by the poorly prepared student who often under adverse conditions rushes through his dissections, oblivious of the fact that one of the chief objects of dissection is to reveal, not to destroy. He often finishes his laboratory work in a few months, and then spends the rest of the year memorizing quiz-compends, studying state-board questions, and taking turns with his teacher in reciting somebody's notes on the subject.

Granted that this minimum requirement has been fulfilled in one way or in another, the question naturally arises whether anything more is desirable as part of the required work of the curriculum. The Committee on Anatomy of the Council of Education of the American Medical Associations, and the Committee of the Association of American Medical Colleges, both recommend that medical students do more than the required dissection, and that this additional work be done in topographical anatomy, by laboratory and recitation methods. At the present day, unfortunately, topographical anatomy is often converted into surgical applied anatomy, and taught entirely out of some such text-book



as above referred to. This is, of course, as unjustifiable as it is undesirable and unfortunate and it is to be hoped that ere long more laboratories in this country will be properly equipped for work in topographical anatomy. If time or circumstances preclude covering the whole of the the body, let the work be confined to regional topographical anatomy, and combine with this laboratory and class work, in which practical relations in general, and such special relations only as can be understood without a knowledge of pathological and surgical conditions are considered. For if it must be a choice between doing much badly and little well, let the preference always be given to the latter. The laboratory can and should supply frozen and dissected sections in all planes, of both sexes and in all ages. The clinic can supply the living individual when needed, and the lecture to a small extent, and mainly the recitation—or better the colloquium—can furnish the occasion for mutual consideration instead of routine drill. Assuredly, topographical anatomy so presented is a wholly different thing from applied anatomy as usually taught.

# THE GENETIC PRINCIPLES OF THE DEVELOPMENT OF THE SYSTEMIC LYMPHATIC VESSELS IN THE MAMMALIAN EMBRYO

PRELIMINARY COMMUNICATION

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THIRTY-FOUR FIGURES<sup>1</sup>

In 1906, at the 22nd session of the Association of American Anatomists, McClure and I presented a joint communication on the development of the main lymphatic channels in embryos of the domestic cat, in their relation to the venous system.<sup>2</sup> In this preliminary paper we held that the lymphatic vessels of the entire mammalian body are formed by the confluence of perivenous mesodermal spaces, developed, as separate anlagen, outside the intima of the early venous channels, but not communicating with the same, except eventually at certain definite points of lymphatico-venous junction which are secondarily formed. This view pronounces for the ontogenesis of lymphatic endothelial cells, lining the separate mesodermal spaces, independently of the pre-existing haemal vascular endothelium. The mesodermal intercellular spaces, thus forming the fundamentals of the future lymphatic vessels, are in no sense derivatives from the embryonic veins, although closely associated with them topographically, and eventually replacing the same.

At the time of the publication of the paper quoted, embodying an outline of these views of mammalian lymphatic ontogenesis,

<sup>1</sup> Cost of illustrations met by the author.

<sup>2</sup> G. S. Huntington and C. F. W. McClure. The development of the main lymph channels of the cat in their relation to the venous system. *Am. Jour. Anat.*, vol. 6, 1907, Abstr. ANAT. REC., vol. 1, pp. 36-41.

McClure and I were not aware of the fact that the mammalian jugular lymph sacs afford, in the typical mammalian organization, in so far as the same is definitely determined at present, the sole or chief portals of entry of the entire systemic lymphatic circulation into the veins.

We consequently failed to recognize correctly the true morphological type of the adult mammalian lymphatico-venous connections in our earlier preliminary paper, and hence regarded them, at that time, as the direct secondary junctions of the independently developed systemic lymphatic vessels with the veins.

The real significance of the adult lymphatico-venous connections was only subsequently recognized by us in the course of a detailed joint investigation of the area involved. A preliminary account of our studies on the development of the jugular lymph sacs in the embryo of the cat, was presented at the 23rd session of the Association of American Anatomists held at Chicago in December, 1907, and published in the Proceedings of that meeting.<sup>3</sup> The details of this investigation, with full critical analysis of all the main developmental stages, in an extensive series of cat embryos, and illustrations of the reconstructions of all important and representative periods, are published in the April number of the American Journal of Anatomy of this year.<sup>4</sup>

After the completion of our joint work on the development of the mammalian jugular lymph sac, I published, in 1907,<sup>5</sup> a genetic interpretation of the development of the mammalian lymphatic system, as a whole, in which I regarded the same as the final product of the union of two genetically different and very unequal components:

1. The entire extensive system of the *lymphatic vessels* of the adult, including the thoracic and right lymphatic ducts and their tributaries, is formed by the confluence of numerous peri-

<sup>3</sup> Geo. S. Huntington and C. F. W. McClure. The anatomy and development of the jugular lymph sacs in the domestic cat. *ANAT. REC.*, vol. 2, pp. 1-18, May, 1908.

<sup>4</sup> *American Journal of Anatomy*, vol. 10, pp. 177-311, April, 1910.

<sup>5</sup> G. S. Huntington. The genetic interpretation of the development of the mammalian lymphatic system. *ANAT. REC.*, vol. 2, pp. 19-45, May, 1908.

venous and extra intimal intercellular mesodermal spaces, in the sense previously defined. These primary anlagen of the future systemic lymphatic vessels are, from their inception, lined by a *lymphatic* vascular endothelium, which is *not* derived from the *haemal* vascular endothelium, but which develops independently of the same.

The lymphatic channels, formed by the subsequent confluence of these originally discrete and separate mesodermal spaces, follow in large part the embryonic veins closely, but they are neither derived from them, nor do they communicate with them, except at the definite points at which the rudimentary mammalian type of a lymphatico-venous heart is developed.

2. This structure develops, as the jugular lymph sac of the typical mammal, directly from the perivenous capillary reticulum of the early pre- and post-cardinal veins, adjacent to, and including, their point of confluence to form the duct of Cuvier.

This mammalian jugular lymph sac, the rudimentary homologue of one of the more highly organized veno-lymphatic hearts of the lower vertebrates, arises directly from the veins. Subsequently, after evacuation of its blood contents, it apparently separates for a short period completely from the same, and finally establishes two sets of permanent connections:

(a) With the independently formed systemic lymphatic channels of the entire body in the majority of the mammalian types carefully determined up to the present date.

(b) Secondary connections with the venous system, re-entering the same at one or more typical and constant points, and thus forming the link which eventually unites the mammalian lymphatic and venous systems, developed primarily independently of each other.

Thus the investigation of mammalian lymphatic development divides itself naturally, in accordance with the postulates of the genetic theory above defined, into three separate and distinct main parts:

1. The development and adult anatomy of the jugular lymph sacs.

2. The development and adult anatomy of the general systemic lymphatic vessels.

3. The mode of union with each other of the two components just enumerated, and the resulting establishment of a continuous *centripetal* lymphatic vascular system, with definite and constant terminals in the venous trunks.

The first of these problems, involving the ontogenetic history of the mammalian jugular lymph sacs, having been established in detail by the joint investigations of McClure and myself above quoted, I intend to follow in similar detail the second and third postulates of the theory of mammalian lymphatic development just outlined, and to prove that, in the composite organization of the final adult lymphatic system, the jugular lymph-sacs, of *direct venous origin*, constitute the links eventually uniting the haemal vascular system of the mammal with the systemic lymphatic vessels, which latter develop *independently of the veins*, by the confluence of numerous intercellular perivenous mesenchymal spaces. The embryonic veins, along and around which the earliest anlagen of the systemic lymphatic channels develop, appear as evanescent and temporary components of the embryonic haemal vascular system. They are not carried into the definite and typical adult venous organization, but they afford, in reference to the correlated lymphatic system, by their separation from the permanent venous channels, and their consequent collapse and atrophy, a series of lines of less resistance in the embryonic body, which paths of easiest progress are utilized by the growing lymphatic vessels. In this way the histological picture of a gradual replacement of an early embryonic vein by a succeeding secondary "perivenous" or "extra-intimal" lymphatic vascular channel is obtained, through the confluence of numerous mesenchymal spaces, surrounding, and eventually replacing, the decadent embryonic veins, but in no sense genetically derived from the latter.

In other words, and in order again to reiterate emphatically the conception of mammalian systemic lymphatic development which I have consistently upheld since my first expression of opinion on the subject, I desire to repeat my conviction that *all*

*systemic* lymphatic vessels of the mammalian embryo, including the thoracic and right lymphatic ducts and their tributaries, are neither in their genesis continuous centrifugal "buds" or "sprouts" from sacs of venous origin, wherever situated, nor "multiple outgrowths" or "veno-lymphatic anlagen," derived from embryonic veins, such "outgrowths" separating subsequently from the veins, and then fusing into continuous and connected lymphatic channels. The systemic lymphatic vessels of the mammalian embryo, as distinguished from the jugular, or reno-caval lymph-sacs, or from any other adult lymphatico-venous junctions of equivalent value, are, on the contrary, in my estimation, from their first ontogenetic inception, structurally and genetically *independent of the haemal vascular system*. Their endothelial lining is *not* derived from the pre-existing embryonic blood vascular endothelium. The multiple independent perivenous spaces forming the anlagen of the future systemic lymphatic channels join to form progressively increasing links of longer channel segments, destined in the normal course of development, to become united into a continuous lymphatic vascular system. This lymphatic system finally attains, in the average and typical mammalian forms, one or more permanent connections with the definite venous system through the portals furnished by the rudimentary lymphatico-venous hearts or lymph sacs. The most prevalent mammalian type of this secondarily acquired lymphatico-venous connection is furnished by the jugular lymph sacs, as outlined in the publications already quoted. While this form of lymphatico-venous junction in the adult is by far the most prevalent type encountered in the mammalian series,<sup>6</sup> there is no reason why, in certain mammalian groups, other points of veno-lymphatic communication, inherited, in these specialized types phylogenetically by selection from the available line of multiple pre-mammalian lymphatico-venous hearts, should not be carried into the adult organization as permanent portals of entry of the lymphatic into the venous system.<sup>7</sup> The post-caval

<sup>6</sup> C. F. W. McClure and C. F. Silvester. A comparative study of the lymphatico-venous communications in adult mammals. *ANAT. REC.*, vol. 3, pp. 534-551, 1909.

<sup>7</sup> G. S. Huntington. The phylogenetic relations of the lymphatic and blood-vascular systems in vertebrates. *ANAT. REC.*, vol. 4, no. 1, January, 1910.

and reno-caval lymphatico-venous connections recently demonstrated by C. F. Silvester<sup>\*</sup> of Princeton University as uniformly found in the entire group of South American primates, and the intermediate correlated conditions found by myself in *Macropus rufus*, are readily and correctly interpreted on this basis.

The present communication is intended as an outline of the development of the mammalian systemic lymphatic vessels, in order to demonstrate what I believe to be the uniform, constant and consistent ontogenetic principle underlying their formation. I have been impressed by the fact that the histological pictures furnished by ungulate, rodent and marsupial embryos are, in reference to the development of the systemic lymphatic channels, relatively obscure and indefinite, when compared with the clear-cut and well-defined conditions encountered uniformly in the aeluroid carnivore. In describing, therefore, in this preliminary account the genetic principle which I believe governs the development of all mammalian systemic lymphatic channels, as distinguished from the lymph hearts of venous origin, I have confined my illustrations to the embryos of the cat, and have selected certain portions of the thoracic ducts of this animal in the critical stages, as concrete examples of the developmental processes occurring in all other regions of the embryo, as will be fully demonstrated in the complete publications to follow. With the ontogenesis of the systemic lymphatic channels definitely established in this form, it is not difficult to determine, by comparison, the presence of corresponding typical developmental conditions in embryos of the pig, rat and opossum. But in none of these latter forms are the typical genetic stages as clearly marked, and the tissues as definitely differentiated as in the cat.

The right and left thoracic ducts develop in cat embryos of between 11 mm. and 16 mm. crown-rump measure. Prior to the 11 mm. stage no anlagen of any portions of the future ducts are observable. In the average 16 mm. embryo the separate anlagen have usually united into continuous lymphatic channels, which

<sup>\*</sup> Twenty-fifth Session of the Association of American Anatomists, Boston, December 28, 29 and 30, 1909.

are connected through the jugular lymph sacs with the systemic veins.

I believe that the adult thoracic ducts of the cat are developed by fusion of three distinct and separate regional segments. Each of these segments is in turn formed by confluence of a number of originally discrete anlagen, which develop independently of the venous system as extra-intimal or perivenous mesenchymal spaces in the sense previously defined (2, 5). These spaces are applied to, or surround, the walls of the embryonic veins of the lower cervical and mediastinal region. The three main divisions, thus formed independently of the venous system, unite with each other to form the channels of the left and right thoracic ducts, and these channels gain their point of entrance into the systemic veins by uniting with a process of the jugular lymph sacs (*thoracic duct approach*) derived from their dorsal aspect, just cephalad to the common jugular approach.

The ontogenetic history of the ducts may therefore be considered under four headings, viz.:

1. *The "Thoracic duct approach" of the jugular lymph sac, forming the terminal of the adult duct on each side.*

2. *The pre-azygos segment.*

This includes two distinct and separate channels:

- (a) *The ventral mediastinal or broncho-mediastinal lymphatic trunk, which drains the ventral mediastinum, viz., the pericardial, tracheal, bronchial, lateral oesophageal and thymic areas.*

This lymphatic channel, associated with the pulmonary arteries, develops through confluence of a large number of separate and independent extra-intimal lymphatic spaces following and surrounding the embryonic venous plexuses of the ventral mediastinum. The chain formed by these spaces eventually unites, directly or indirectly, with the similar chain forming the anlage of the pre-azygos segment of the thoracic duct.

- (b) *The pre-azygos segment of the thoracic duct includes the portion of the main channel from the point of its entrance into the jugular lymph sac, through the thoracic duct approach of the latter, caudad to its intersection with the dorsal surface of the aortic arch.*



In the adult animal this segment forms the relatively long portion which ascends cephalo-sinistrad from the point where the duct paræs company with the right azygos vein, under cover of the aortic arch, and the vertical portion of the left subclavian artery, dorsal to the vertebral vein and to the left innominate confluence, to its junction with the jugular lymph sac. In this part of its course the thoracic duct receives the lymphatic return from the ventral mediastinum through channels which join it to the ventral mediastinal trunk as just defined. The pre-azygos segment of the main duct is again formed in the embryo by confluence of independent mesenchymal spaces around and along the prevertebral and dorsal mediastinal venous plexuses of the embryo.

3. *The azygos segment* comprises the portions of the thoracic ducts caudal to the level of the aortic arch. It develops, again independently, as the result of fusion of a number of extra-intimal mesenchymal spaces closely applied to the ventral surface of the azygos veins, and of their ventro-medial tributaries, or surrounding the latter.

4. *The post-azygos segment*, through which the thoracic ducts establish their connection with the Receptaculum and the system of the abdominal lymphatics.

The purpose of the present paper is to employ the facts ascertained in regard to the development of the two thoracic ducts as a concrete illustration of the genetic principles underlying the formation of all systemic lymphatic organization.

For this purpose the right and left ducts will be regarded as bilateral equivalents, as they actually are in certain stages. As a matter of fact the right channel in the main azygos region is the first portion to differentiate clearly and offers the best illustration of lymphatic histogenesis in the earlier and critical stages.

Inasmuch as the development of the post-azygos segment of both ducts is intimately connected with that of the principal abdominal lymphatic channels, and hence requires for its elucidation a detailed consideration of these structures, I will confine my illustrations in the present paper to the development of the two main anterior segments, viz., the pre-azygos and the azygos portions of the entire duct, with the distinct understanding that

identical ontogenetic processes are responsible for the development not only of the post-azygos segments of the ducts and the mesenteric lymph sacs, but for all other systemic lymphatic channels of the entire body.

## I. PRE-AZYGOS SEGMENT OF THE THORACIC DUCT

### A. *Ventral or broncho-mediastinal trunk*

The area in which this lymphatic channel develops, is shown topographically in fig. 1, a transverse section of the upper thoracic region in a 12 mm. embryo (series 78, slide 5, section 9.) The lymphatic anlagen arise in the mesenchyme between the pulmonary arteries (10) ventrally, the coelom laterally, the precardinal veins (3, 6), vagi (22), trachea (9) and aorta (7) dorsally. This area is indicated by the *x* in fig. 1.

In the earlier stages (embryos between 11 mm. and 14 mm.) an extensive ventro-medial capillary network obtains along and between the main venous lines of the right and left sides, involving the caudal part of the internal jugular, the common jugular and innominate veins.

Now, if the ventral portion of this venous plexus is followed caudad into the upper thoracic region, the following observations can be made in stages of the proper length, and adequately fixed and stained:

(1) In embryos between 11 and 12 mm. only venous capillaries are found, in the majority of cases.

(2) In 13 mm. embryos certain of the venous radicles entering into this plexus are partly surrounded and enveloped by independently developed extra-intimal lymphatic spaces, the first anlagen of the future ventral mediastinal lymphatic channel.

Fig. 2 shows a section of this region in a 13 mm. embryo (series 107, slide 9, section 40).

Between left pulmonary artery (10) and aorta (7) are branches of the ventral mediastinal plexus. One of these (4) is partially surrounded by a lymphatic anlage (5), but the process of replacement is in its earliest phases.

(3) In the 13.5 mm. embryo the full and convincing proof of the extra-intimal derivation of this channel is given.

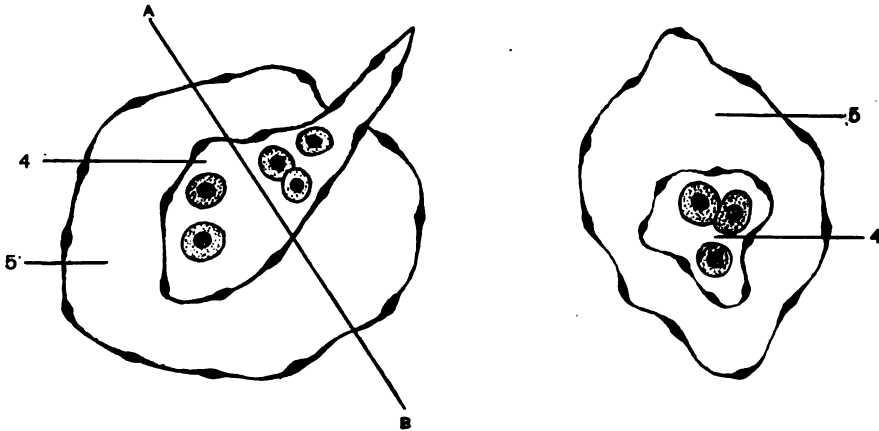
Fig. 3 shows a transverse section of the upper thoracic region of a 13.5 mm. embryo (series 189, slide 8, section 36). Just ventromesad of the left vagus nerve and its encircling vein is a venous radicle (4) almost completely surrounded by an extra-intimal lymphatic space (5) in the process of replacing the atrophying vein with which it is so closely associated. The corresponding structures are seen on the right side (4, 5).

Fig. 3A shows the extra-intimal lymphatic space and the contained vein on the left side of this section in a higher magnification ( $\times 300$ ). It will be seen that the lymphatic space nearly envelops the venule. The latter, if followed cephalad and caudad, is found separated from the functional venous channels. It appears collapsed and shrunken, and contains only a few degenerating erythrocytes. We are dealing here with a further advance in the conditions found in the immediately preceding 13 mm. stage. (Fig. 2, series 107, slide 9, section 40). The venous core of the earlier lymphatic anlage is in process of further recession and degeneration, as the perivenous lymphatic space enlarges and more and more completely replaces the antecedent venous channel upon and around which it develops. On the right side of fig. 3 (series 189, slide 8, section 36), the section has cut the corresponding vein and the enveloping extra-intimal space at right angles, so that the central kernel of the shrinking vein (4), still containing a few red blood cells, is nearly surrounded by the replacing extra-intimal lymphatic (5). The vein, or rather its remnant, bears a relation to the perivenous replacing lymphatic which is exactly the same as that of a collapsed inner tube to the enveloping shoe of a pneumatic tire. The inner skin of the shoe and the rim of the wheel represent the *lymphatic intimal endothelium*. The space between them and the collapsed inner tube is the *lumen* of the future *ventral mediastinal lymphatic channel*. The inner tube itself is the *embryonic vein* upon which the secondary lymphatic channel is built. In the course of further development it disintegrates and disappears, leaving a clear lumen to the lymphatic channel which thus secondarily replaces it.

Usually the replacing lymphatic begins as an extra-intimal chan-

nel *partially* surrounding the embryonic vein which it is destined to replace. This leads in the course of further development to an expansion of the lymphatic space not concentric with the axial line of the shrinking vein. The remnant of the vein retires to a point on the intimal circumference of the new lymphatic channel and appears to project into the latter.

The resulting histological pictures are hence in many cases quite analogous to the appearance of a mesonephric glomerulus in its relation to the lumen of a Wolffian tubule. Of course, as in the case of this illustration, a section, for example, in the axis of the line A-B will divide the shrinking vein and the enveloping lymphatic in such a way as to produce the following picture:



This, however, is exceptional.

This is not a haphazard process, observed only occasionally, in a limited number of embryos, and then only in single sections, or, at most, in a few successive sections. In any average embryo of the proper length the same structures appear in the same situation and in identical relationship to the embryonic environment. It is often possible to follow the forming lymphatic with its atrophied vein kernel for long distances, and in different embryos of the same crown rump measure the constant repetition of identical histological pictures is remarkable.

There are, of course, individual cases of variation, in which systemic lymphatic development is either more advanced or more retarded than is normal for the average run of embryos in a given stage. But if a very large number of embryos of each typical period are examined and compared the *average* stage of extraintimal lymphatic development attained by the majority of individuals in each period is remarkably constant and uniform. I shall have occasion, in the complete publication, to refer again in detail to the question of chronological embryonic variation.

The existence of the perivenous lymphatic spaces in this and other regions of the embryo has been so often denied by recent contributors to the subject, or, if admitted, explained in every possible way except on the basis of the correct interpretation, that I publish in this paper a series of micro-photographs of five successive sections through the pretracheal mediastinal region of a 13.5 cat embryo (series 189, slide 8, sections 36 to 40) (figs. 3 to 7).

Fig. 3, above described, shows the general topographical area involved. Figs. 4 to 7 are cut down to economize space.

In all five figures the atrophying vein kernel (4) and the replacing lymphatic anlage surrounding the same (5) have been cut obliquely on the left side of the embryo, and hence give longer stretches of the structures (4 and 5) involved. On the right side the plane of section is more at right angles to both the venous core and the enveloping lymphatic space in the first four figures. In fig. 7 the lymphatic space of the right side terminates in characteristic fashion blindly and the atrophied vein merges imperceptibly into the surrounding mesenchyme. The remnants of partially degenerated erythrocytes in the lumen of the atrophied venous core are especially clearly seen in all the sections on the left side.

Of course the photographs, and especially the reduced reproductions, offer far less striking histological pictures than the stained and differentiated slide, although they sufficiently well demonstrate the actual conditions.

In the illustrations only a few of the more marked areas of lymphatic replacement of decadent venules are indicated by the

leaders 5 and 4 respectively. Numerous other smaller areas of identical significance are seen on close examination in adjacent parts of the field.

In the succeeding 14 mm. stage the ontogenetic process just outlined is, in the average embryo of this measure, fully developed.

Fig. 8 shows a section of a 14 mm. embryo in this region (series 214, slide 13, section 13). Comparison with fig. 3 will show the existence of the identical relations between the same decadent vein and the replacing extra-intimal lymphatic on both right and left sides. The embryos are cut approximately in the same plane and hence the resulting pictures are almost identical.

Figs. 9, 10, 11, and 12 show corresponding sections of the same embryo further caudad.

In fig. 9 three areas are indicated by leaders in which the atrophied vein (4) is in relation with the enveloping and replacing extra-intimal lymphatic anlage (5). In the succeeding section (fig. 10) the two dorsal areas have practically become confluent, and the tortuous and collapsed endothelial bag representing the remnant of the decadent venule (4) can be followed for some distance. The ventral area in fig. 9 offers only an indistinct central venous core (4), surrounded by the lymphatic anlage (5). In the succeeding section (fig. 10), however, the unmistakable relationship and significance of the two spaces is clearly revealed.

The two successive sections of the same slide of this embryo, shown in figs. 11 and 12, give remarkably distinct histological pictures of lymphatic ontogenesis, and also show the gradual increase in the area of the lymphatic perivenous compartment as compared with the contained venous remnant. In both sections a few red blood cells are still to be noticed within the lumen of the latter.

Finally, in another 14 mm. embryo (figs. 13 and 14, series 212, slide 10, sections 5 and 6) conditions identical with the preceding are well shown on both sides of two successive sections. The same decadent venules (4) and the associated enveloping perivenous lymphatic anlages (5) are found in the typical situation between trachea, aorta and vagi dorsad and the pulmonary arteries ventrad.

Fig. 14 likewise offers the explanation of the fact that the average 14 or 14.5 mm. embryo affords the clearest and most distinct pictures of systemic lymphatic ontogenesis. In these stages the decadent vein (4), detached from the functional venous channels, is still relatively large, while the perivenous lymphatic space (5) has also markedly increased in size as compared with the 13 mm. stage. The two structures, taken together, form therefore striking histological objects in the field. Subsequently, with the further degeneration and final complete elimination of the venous kernel, and the condensation of the perivenous lymphatic space into a definite lymphatic channel, the lumen of the latter appears relatively smaller. Thus in two successive sections of a 15 mm. embryo (series 216, slide 10, sections 32 and 33, figs. 15 and 16) the identical lymphatic anlage (5 in figs. 15 and 16) can readily be traced, but appears now as a wide channel with clear lumen. The central venous core, so prominent in the earlier stages (13, 13.5 and 14 mm.) has either disappeared entirely, or is merely indicated by insignificant remnants (4). The same conditions, with further condensation of the mesenchyme, and consequent further reduction of the lymphatic lumen, are encountered in the 15.5 and 16 mm. stages (fig. 17, series 215, slide 14, section 13, 15.5 mm. and fig. 18, series 230, slide 12, section 25, 16 mm.)

No impartial observer can mistake the significance of the conditions here shown. Every stage of the process can be followed in detail. The behavior of the decadent embryonic vein, and its relation to the enveloping extra-intimal lymphatic channel, are absolutely demonstrated. The endothelium of the shrinking vein has no share in furnishing the independent lymphatic endothelium of the replacing mesenchymal space, and nowhere, in the entire process, is there the faintest suggestion of an "out-bud" or of a "splitting off" from the circumference of an otherwise valid embryonic vein of "lymphatic" or "veno-lymphatic" anlages.

The conditions here described are definite ontogenetic *facts* remarkably constant in every embryo of the proper age. They cannot be disregarded in promulgating *theories* of mammalian lymphatic development. The only conclusion which seems to

me to be warranted by actual observation is that certain embryonic veins form, during the process of their atrophy and final elimination from the definite venous organization, the supporting lines along which certain of the perivenous extra-intimal lymphatic anlagen first develop. The initial development of lymphatic spaces, is, however, by no means *confined* to the immediate environment of a degenerating embryonic vein. The same field which demonstrates the histogenetic processes above described in the development of the extra-intimal lymphatic spaces surrounding a decadent vein will, at the same time, show numerous equivalent lymphatic spaces developing independently of antecedent veins as enlarging intercellular mesenchymal clefts.

These early lymphatic anlagen, formed independently of antecedent embryonic venous capillaries, are smaller and offer less striking pictures, than those which develop in association with an atrophying vein, and which hence reach a greater size at a relatively early period. They are more difficult to differentiate, but their existence can on close examination be absolutely determined, and their connection with the larger perivenous lymphatic spaces can be established.

The fact that numerous early embryonic venous channels, large and small, atrophy and disappear during the normal course of subsequent development, appears to afford a more favorable field for the greater development of the adjacent mesenchymal intercellular spaces, so that these enlarge more rapidly, as the correlated vein recedes. This relationship appears, however, to be based exclusively on the physical and mechanical advantages which the abandoned and shrinking primary venous line affords to the adjacent mesenchymal spaces for more rapid enlargement in the sense of replacing the disappearing vein and occupying secondarily the space formerly filled by the haemal channel. This is evidently an important factor in determining the size and extent of the final lymphatic channel resulting from the confluence of the originally separate and independent perivenous anlagen. Consequently, in the adult, the largest and best defined systemic lymphatic vessels either accompany reduced adult remnants of a relatively larger embryonic venous channel, or, in case



of the latter's entire default, topographically replace the same. Now, while this relation manifests itself strikingly in many parts of the body, it is quite evident that the development of lymphatic channels occurs in other parts independently of preceding veins, by the confluence of independent intercellular mesenchymal spaces.

In judging regarding the genetic principles underlying mammalian systemic lymphatic development it is absolutely necessary clearly and correctly to value the relations above detailed between degenerating early embryonic venous channels and the systemic lymphatic anlagen developed in association with them and destined to eventually replace them more or less completely topographically. I can readily see why certain recent contributions to the subject assume that the well defined lymphatic channels of a later stage are the direct derivatives of the equally well defined venous plexuses of earlier embryos, since they cover each other mutually absolutely in the topographical sense. Such an assumption is, however, in my opinion, faulty, because it is based on insufficient or inaccurate observation, and fails in correctly interpreting the genetic factors responsible for the topographical replacement of an earlier vein by a later lymphatic channel.

Again, a careful consideration of the facts above detailed, must inevitably lead to the conviction that the real developmental processes active in systemic lymphatic ontogenesis can never be determined by injection of embryos however successful. A glance at the preceding illustrations will show that a successful injection of the embryonic venous system might very well, before complete detachment has occurred, fill from the permanent haemal channels the still large and patent portions of the venous plexus already for the most part surrounded by the extra-intimal lymphatic anlagen. Such a preparation would lead the observer to conclude that the line of the future lymphatic channels was still altogether venous. He would have no means of determining the co-existing true lymphatic anlagen, nor could these be demonstrated by a simultaneous lymphatic injection, because, at this period, they are isolated segments of the future lymphatic chain, not yet in communication with each other, or with the veins through the jugu-

lar lymph sacs, or with any other channel system, from which they could be filled. Subsequently, when the continuity of the systemic lymphatic vessels has been established, and can be demonstrated by injection, the site of the former venous plexus is occupied by lymphatics, but the conclusion that these are the former veins, directly transformed into lymphatics, is just as erroneous, as the same conclusion based on the examination of serial sections in different stages, in which the *topographical* replacement of the earlier vein by the later lymphatic is taken as the only criterion, and as affording proof of their genetic identity.

In view of the facts absolutely established by direct and repeated uniform observation in embryos of *Felis domestica*, it seems to me that it is worth while to examine the available evidence here offered in this form carefully and impartially, rather than torture an interpretation into mammalian lymphatic ontogenesis which is not supported by the actual conditions found in embryos of this specific mammalian type.

The cat may differ in its details of lymphatic development and in its adult lymphatic organization from the conditions obtaining in certain other mammalian types, as yet imperfectly determined. And yet these differences, established and maintained within the natural limits of the mammalian class, cannot, if they actually exist, be *basic*. In any given individual mammalian form, the systemic lymphatic vessels, whatever their adult relation to and connection with the venous system may be, must develop in accordance with a genetic ground plan common to all mammalia.

*B. The development of the proximal portion of the thoracic duct proper, between the termination of the thoracic duct approach of the jugular lymph sac, and the beginning of the azygos segment of the thoracic ducts, caudal to the level of the aortic arch.*

In the earlier purely venous stages a venous plexus between oesophagus and vertebral column drains caudo-laterad into the mesal surface of the main jugular and innominate trunks. This plexus continues the supra cardinal line cephalad beyond the

level of the azygos-Cuvierian junction. The terminals of this plexus are frequently joined by dorsal somatic venous tributaries near their entrance into the main vein.

Some of the elements of this early embryonic prevertebral venous plexus are secondarily replaced by perivenous or extra-intimal lymphatic spaces in exactly the same way as in the development of the ventral mediastinal duct. The resulting, originally separate, extra-intimal lymphatic anlagen, having replaced the venule along and around which they developed, unite with each other and form the pre-azygos segment of the thoracic duct, between the thoracic duct approach of the jugular lymph sac and the level of the aortic arch, at which the azygos portion of the thoracic ducts begins.

The general area in which this development proceeds, is indicated in the topographical fig. 1, by the letter Y.

The embryonic stages between 13.5 and 15.5 mm. furnish abundant evidence of this genetic process. Figs. 19 and 20 show two sections of a 14 mm. embryo (series 210, slide 9, sections 23 and 26) in the prevertebral area of the upper thoracic region. The anlage of the pre-azygos segment of the thoracic duct (5) is seen on the left side of the interval between oesophagus and the prevertebral plexus (17) and sympathetic nerve(1). The sections show the identical characters previously noted in the development of the broncho-mediastinal trunk, but both the decadent central venous core of the anlage (4) and the perivenous lymphatic space (5) are larger and better developed.

These pictures are again constant in embryos of the appropriate stages. The lymphatic anlage can be accurately traced from its indefinite beginning among the perivenous mesenchymal intercellular clefts through a number of successive sections to its similar distal termination in the same intercellular plexus. Following the sections from this point caudad through a varying intervening area in which no distinct lymphatic channel appears, the same line will sooner or later reveal the repetition of the same process, and the formation of another link in the still disjointed chain of primitive lymphatic anlagen, eventually destined to unite into the continuous-channel of the pre-azygos segment of the thoracic duct.

## II. THE AZYGOS SEGMENT OF THE THORACIC DUCT

This main part of the thoracic duct develops by the confluence of the extra-intimal lymphatic anlagen, which begin to appear in the 12.5 mm. embryo, are clearly marked in the 13 and 13.5 mm. embryo, increase in the 14 mm. stage, become confluent to form longer segments in the 15 and 15.5 mm. embryos, and finally unite into the bilateral and continuous channels of the thoracic ducts in the average 16 mm. embryo, although instances are not rare in which the complete continuity of the thoracic ducts is not attained until a later stage. These extra-intimal lymphatic anlagen develop in close association with the ventral aspect of the azygos veins and their ventral branches, but are from the beginning genetically distinct and independent of the same.

In the earlier and purely venous stages, the azygos veins receive, in addition to the terminals of the supracardinal plexus, larger dorsal somatic tributaries from the body walls and from the interior of the vertebral canal, and smaller ventromedial branches which drain the periaortic space close to the wall of the main arterial vessel. When these ventral azygos tributaries appear they occupy in general the position described by McClure as characteristic for the cardinal collateral plexus of the Marsupalia.\*

The ventro-medial azygos tributary plexus is found in the purely venous condition, before any perivenous lymphatic development associated with it has begun in this region, in embryos of 11 and 12 mm. (Fig. 21, series 213, slide 11, section 29, 11 mm.; fig. 22, series 217, slide 11, section 27, 12 mm.). The plexus occupies the area ventral to the intersegmental aortic branches and the sympathetic nerves, between the aorta and the main azygos trunks.

Later, in 13.5 to 14 mm. embryos, portions of this early plexus appear detached in certain areas of the sub-azygos region from the main venous trunks. In many cases the line of the obliterated connection can still be traced for a time as a strand of differentiated mesoderm, and the separated elements of the azygos plexus still

\* C. F. W. McClure. The anatomy and development of the post-cava in *Didelphis marsupialis*. *Am. Jour. Anat.*, vol. 5, 1906.

contain frequently red blood cells in the earlier stages. The lymphatic anlagen of the thoracic ducts form along and around these degenerating elements of the azygos plexus, as extra-intimal or perivenous spaces, in exactly the same manner as above described for the regions further cephalad.

The recognition of this reduced ventro-medial tributary system of the azygos veins is of the greatest importance to the correct interpretation of the mammalian thoracic duct development. Not only do the extra-intimal lymphatic anlagen of the azygos segments of the duct form along and around these venules, but in the same way the anterior part of the mesenteric lymphatic network of the abdomen has its origin in the extra-intimal lymphatic spaces which develop around the caudal continuation of the ventral plexus in front and along the sides of the abdominal aorta, in the root of the dorsal mesogastrium. These perivenous lymphatic spaces subsequently unite to form the receptaculum and establish, on one hand, connections with the independently developed intestinal lymphatic channels, and, on the other, with the thoracic duct.

McClure, in a paper published in 1908,<sup>10</sup> on the development of the thoracic ducts in the cat, very clearly described and figured this secondary and evanescent line of the venous capillary plexus along the innominate and azygos veins which forms the basis for the subsequent development of the main segments of the thoracic duct. I can completely confirm the accuracy of his observations on this structure, which he for the first time mapped out and demonstrated completely. I am obliged to differ from him, as shown in the preceding pages, in reference to the interpretation of the rôle taken by the temporary venous plexus in the development of the thoracic ducts. I cannot regard the ducts as arising directly from the detached venous elements of the plexus, but believe, as here shown, that these elements are secondarily replaced by independent extra-intimal lymphatic spaces, which then join to form the continuous channels of the thoracic ducts.

<sup>10</sup> C. F. W. McClure. The development of the thoracic and right lymphatic ducts in the domestic cat. *Anat. Anz.*, Bd. 32, nos. 21 and 22, 1908.

I am quite convinced that, in determining definitely questions as intricate as are the relations between developing haemal and lymphatic channels in the mammalian embryo, a very large number of individual examples of each stage are absolutely necessary. I feel that if McClure had had at his command the amount of material on which this communication is based, his conclusions would have coincided with those here expressed, and he would not have assigned to the thoracic ducts a genetic origin different from that which we upheld for all systemic lymphatic development in the mammalian embryo in our first joint publication on the subject in 1906 (2), and which, with the exception of the thoracic ducts, he still regards as the fundamental basis of systemic lymphatic development.

It is necessary to exercise great care in the critical stages in order correctly to distinguish between the degenerating vessels of the plexus and the extra-intimal lymphatic anlagen replacing them, and to compare results obtained from a number of embryos of the same stage. If this is done there can remain no doubt that the azygos segments of the two thoracic ducts in the embryos of the cat develop by confluence of extra-intimal perivenous lymphatic spaces. These anlagen appear at first as isolated spaces, either surrounding the retreating veins or closely applied to part of their circumference and subsequently to the ventral wall of the main azygos trunks, usually laterad to the points where the ventral plexus connects with the main azygos channel. Thus, compare the micro-photographs of series 34 and 214, figs 23 to 32. In the succeeding stages these numerous separate lymphatic anlagen coalesce into longer continuous channel-segments. It is again noteworthy that in stages between 13.5 mm. and 14 mm., the still separate and distinct lymphatic anlagen are relatively larger and more clearly evident than in the subsequent (15 mm. to 15.5 mm.) stages in which they have more extensively joined to form longer links of the system. Finally, in the 16 mm. embryo, where usually all the separate segments are assembled into the continuous channel of the thoracic duct, additional new mesenchymal spaces are added and thus a second and permanent increase in size and caliber of the latter appears to begin, which

can be traced in the subsequent stages as occurring in correlation with the reduction of the adjacent azygos trunks. The mammalian systemic lymphatic vessels seem to thus share with the embryonic veins this tendency towards apparently excessive diffuse plexiform development in their respective early genetic stages. Subsequently the definite channel, lymphatic or hæmal, seems to concentrate along static lines, as a vessel of relatively smaller caliber, out of the antecedent more generalized plexus, and from this stage on further growth centres on the definite vessel replacing the earlier diffuse plexus.

In a 14 mm. embryo (series 34, Princeton Embryological Collection, slide 31) the main azygos trunks have increased in size, have approached the dorso-lateral circumference of the aorta more closely, and the interazygos anastomosis has developed.

The ventro-medial plexus is, however, still present in the typical position—(figs. 23 and 24, 4; series 34, slide 31, sections 18 and 19.)

Further cephalad (slide 28 of the same embryo), the beginning extra-intimal replacement of this plexus by the lymphatic anlagen of the thoracic duct is encountered (figs. 25 and 26, series 34, slide 28, sections 19 and 20). The venule (figs. 25 and 26, 4), still containing a few red blood cells, is almost completely detached from the definite azygos venous channel, although its original continuity with the same can still be traced by a strand of differentiated mesenchyme representing the obliterated channel of communication. This central detached venous kernel (4) is surrounded by the extra-intimal lymphatic space (5).

Figs. 27 to 30 show successive sections from two slides of another 14 mm. embryo (series 214), in which the process of lymphatic replacement of the azygos plexus is further advanced. All trace of the original connection of the central venous core (4) with the azygos system is lost in these sections. The detached and abandoned venule is entirely empty and forms a partially collapsed endothelial tube surrounded, as before, by the perivenous lymphatic anlage of the thoracic duct (5). The four figures show this development both at the level of the inter-segmental arteries (figs. 27 and 28) and in the intervals between these aortic branches (figs. 29 and 30). The figures published here are not isolated sec-

tions to which the conditions described are confined, but the same structures extend cephalad and caudad for a considerable distance. The same embryo again shows, further caudad, admirably the first inception of the lymphatic anlage in relation to the ventro-medial azygos plexus. (Figs. 31 and 32). The vein undergoing lymphatic replacement (4) is detached from the remainder of the plexus, but the original connection is still indicated. The lymphatic space (5) has developed, as yet, only on the lateral aspect of the abandoned vein, and has not yet completely enveloped the same. Comparison with the sections further cephalad, especially with figs. 27 and 28, at the intersegmental arterial level, clearly indicates that in course of further development additional portions of the ventro-medial venous plexus will be involved and included in the enveloping extra-intimal lymphatic, and that, in attaining this condition, the thoracic duct anlage will extend relatively further dorsad and thus come into closer apposition with the main azygos trunks.

I am bound to draw, from the observations here recorded, the following conclusions:

1. The pre-azygos and azygos segments of the thoracic ducts of the cat are formed by confluence of separate and independent lymphatic anlages, which develop from intercellular clefts in the prevertebral mesenchyme. A large proportion of these early lymphatic anlages develop as extra-intimal para- or peri-venous mesenchymal spaces along the early mediastinal and azygos plexuses and their tributaries.

2. These spaces, whether developed directly in the mesenchyme, or in association with regressive embryonic veins, are from their first inception independent mesenchymal intercellular clefts. Their origin is independent of the veins which they are subsequently to replace topographically. They are neither "buds" derived from the veins, nor are they portions of the primitive veins separated or "split off" the main channels. Their lymphatic intimal endothelial lining develops with their first appearance from the indifferent mesenchymal cells lining the spaces, and is the result of the adaptation of these cells to the new mechanical and physical conditions imposed on them by the space formation.



The lymphatic endothelium does not arise by "sprouting," or otherwise, from the pre-existing haemal vascular endothelium of the early embryonic veins.

As a matter of fact, in place of being derived from the endothelium of the blood channels, the intima of the degenerating vein can in hundreds of observations be followed through its stages of disintegration, partial reversion to indifferent mesenchymal cells, and final complete elimination, within the lumen of the extra-intimal lymphatic channel partly or completely enveloping the venous rudiment. Nowhere is there the slightest indication of "budding" or "sprouting," or of any other active process on the part of the degenerating haemal endothelium.

3. The above named individual segments of the thoracic and right lymphatic ducts, thus formed through confluence of a large number of separate and independently developed mesenchymal and perivenous anlagen, finally unite with each other to form a continuous bilateral channel, which secondarily effects a junction with the thoracic duct approach of the jugular lymph sac, through which the general lymphatic system gains its entrance into the venous system.

4. The thoracic ducts, especially in their pre-azygos and azygos segments, and in the area of the tributary ventral mediastinal trunk, offer the most striking and convincing evidence of the truth of the extra-intimal theory of systemic lymphatic development in this mammalian embryo, in the relation exhibited by the first perivenous lymphatic anlagen to early embryonic venous channels which they surround and subsequently replace.

For this reason I have selected the thoracic ducts as representative systemic lymphatic channels, whose developmental history will serve as a concrete illustration of the genetic principles expressed in this communication. Many of the details of the thoracic duct development are here designedly not considered, although they are of great importance and significance.

These questions can be much more clearly and comprehensively studied in their relation to the adult anatomy of the ducts and their mode of union with the system of the abdominal lymph-

phatics. They will be considered in detail in a more extensive memoir on mammalian lymphatic development to be presently published.

As above stated, the development of parts of the thoracic ducts is introduced in this paper solely for the purpose of affording a concrete illustration of the general principles underlying the development of all the systemic lymphatic channels in the particular mammalian embryo (*Felis domestica*) here considered. The same principles obtain in systemic lymphatic genesis in all mammalian types which I have had the opportunity of examining, but the embryos of the cat offer by far the most conclusive, consistent and striking evidence.

5. The early independent genetic history of the spaces, which I have above described as the first anlagen of the thoracic duct channels in the embryos of the cat, and the fact that in subsequent stages they appear consistently and in every possible combination as extra-intimal or perivenous mesenchymal spaces, following and surrounding the branches of the prevertebral, ventral mediastinal and ventro-medial azygos venous plexuses, excludes to my mind, the possibility of considering them as *direct* derivatives from the venous plexuses, or as so-called "venous outgrowths" of the innominate and main azygos veins, subsequently detached from the parent trunks. The actual conditions observed and here described are too obvious and constant to admit of any doubt. They can be verified by any observer on sufficient material of the proper stages. I think it is time for investigators engaged in solving the problem of mammalian lymphatic development to abandon superficial lines of comparison and generalization, based often on isolated and insufficient observations, or, as in the injection experiments, on methods which, from the nature of the problem, are utterly inadequate and almost barbarous. Results obtained from observations of this kind are, at their best, misleading, when dealing with a genetic question as delicately balanced as is the relation between developing haemal and lymphatic channels in the mammalian embryo.

## EXPLANATION OF FIGURES

The series here figured and described are in the embryological collection of Columbia University, with the exception of series 34, which belongs to the embryological collection of Princeton University. I am greatly indebted to Prof. C. F. W. McClure for the opportunity of studying this series and of publishing the four sections shown in figs. 23 to 26.

### ANNOTATION OF LEADERS IN ALL FIGURES

- |   |                                       |
|---|---------------------------------------|
| 1 Sympathetic nerve.  | 17 Prevertebral venous plexus.        |
| 2 Intersegmental arteries.  | 21 Thymus.                            |
| 3 Precardinal, resp. azygos vein of<br>right side.                                    | 22 Vagus.                             |
| 4 Degenerating vein.  | 23 Carotid artery.                    |
| 5 Extraintimal or perivenous lymphatic space surrounding degenerating embryonic vein. | 24 Thyrocervical artery.              |
| 6 Precardinal, resp. azygos vein of<br>left side.                                     | 25 Internal jugular vein.             |
| 7 Aorta.  | 26 Common jugular vein.               |
| 8 Oesophagus.   | 31 Primitive ulnar veno-lymphatic.    |
| 9 Trachea.  | 32 Ventral mediastinal venous plexus. |
| 10 Pulmonary arteries.  | 33 Subclavian artery.                 |
| 16 Dorsal somatic tributaries.  | 40 Innominate vein.                   |
|   | 48 Right auricle.                     |
|   | 49 Left auricle.                      |
|   | 50 Right ventricle.                   |

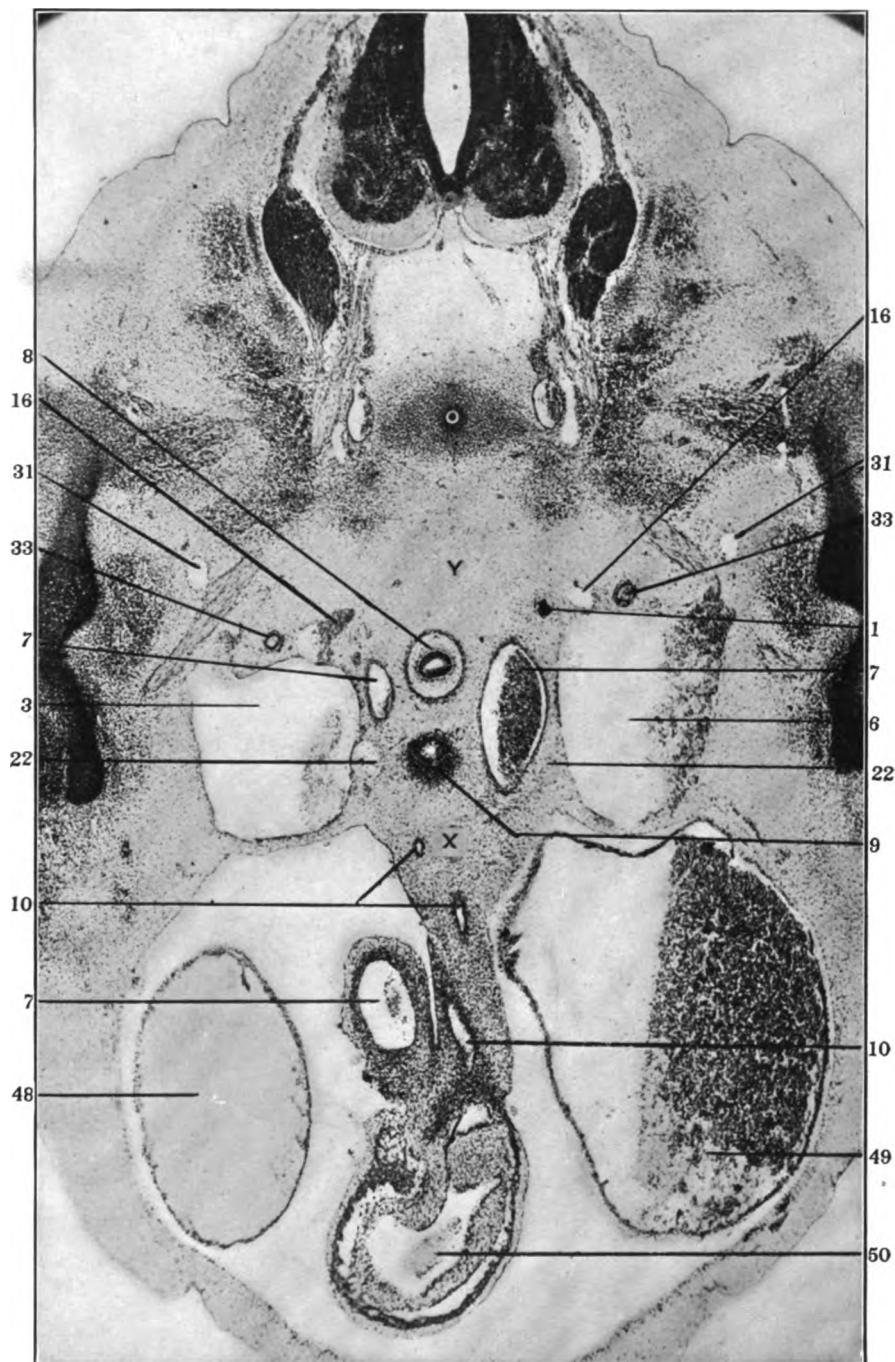


Fig. 1 Transverse section of anterior thoracic region of 12 mm. cat embryo (series 78, slide 5, section 9,  $\times 50$ ).

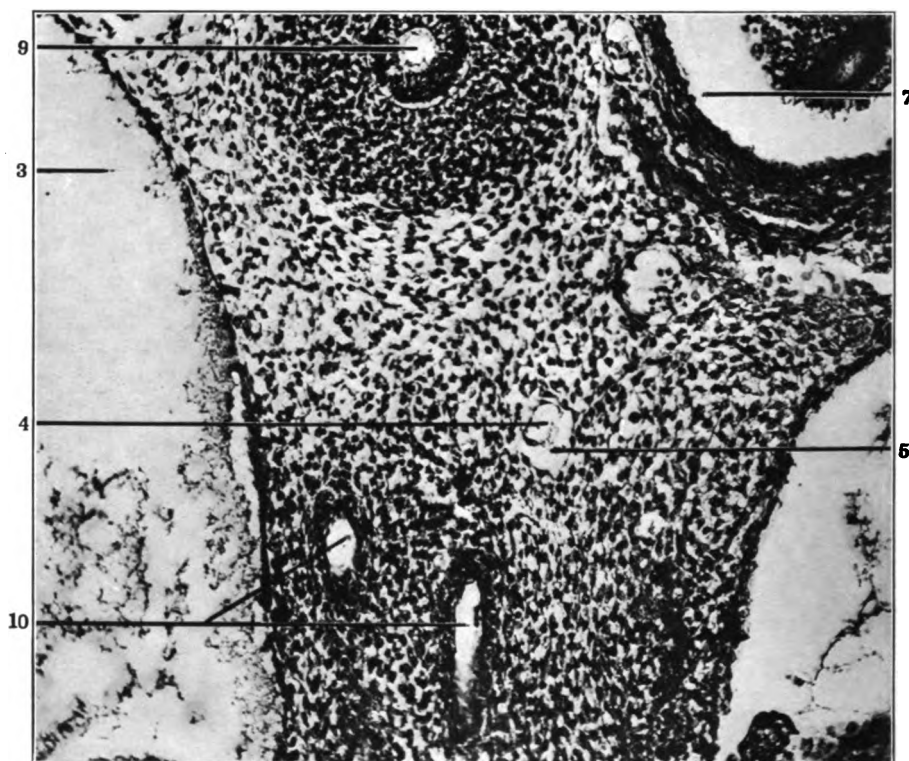


Fig. 2 Transverse section of anterior thoracic region of 13 mm. cat embryo (series 107, slide 9, section 40,  $\times 225$ ).

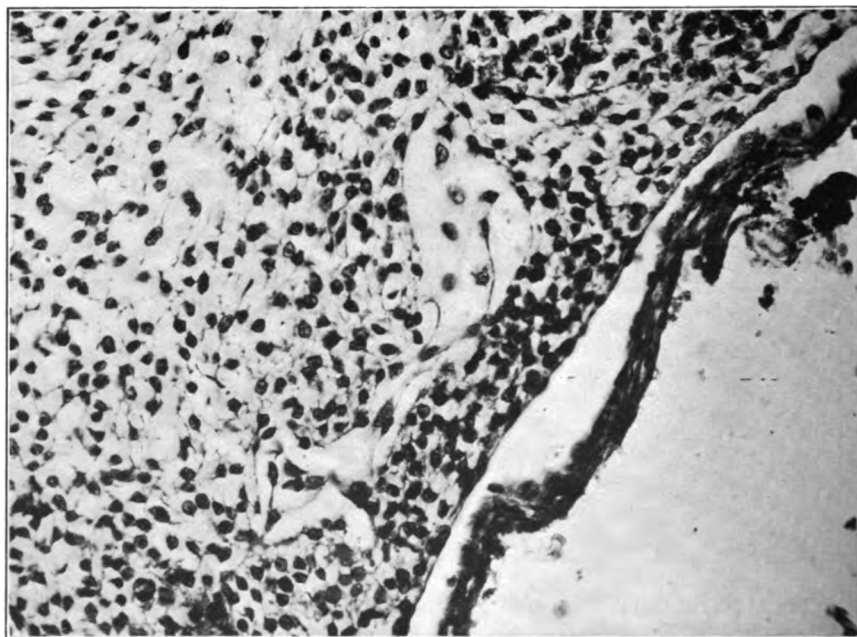


Fig. 3a Extra-intimal lymphatic anlage and contained atrophied vein of same section as fig. 3, magnified 300 diameters.

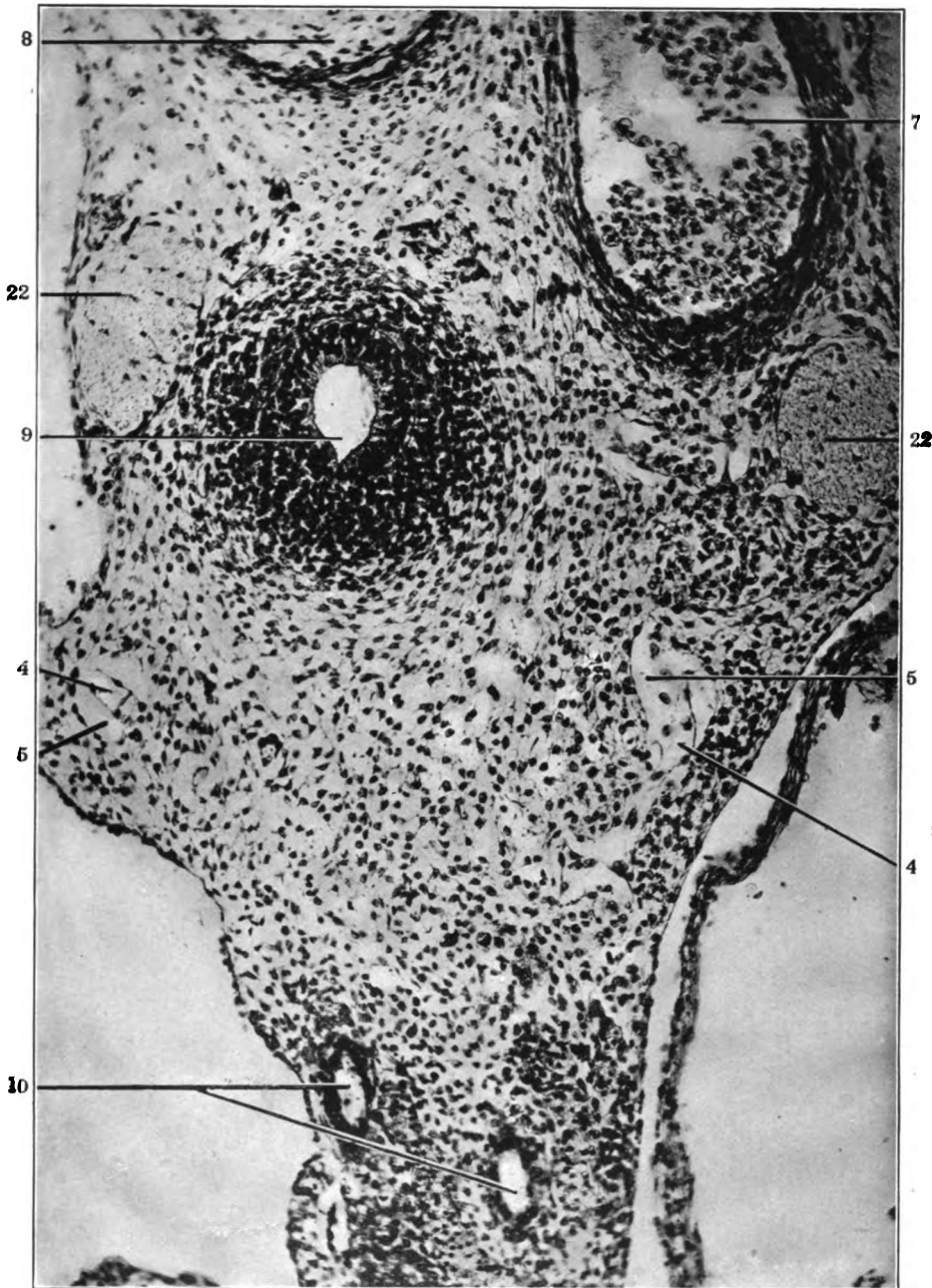


Fig. 3 Transverse section of anterior thoracic region of 13.5 mm. cat embryo (series 189, slide 8, section 36,  $\times 225$ ).

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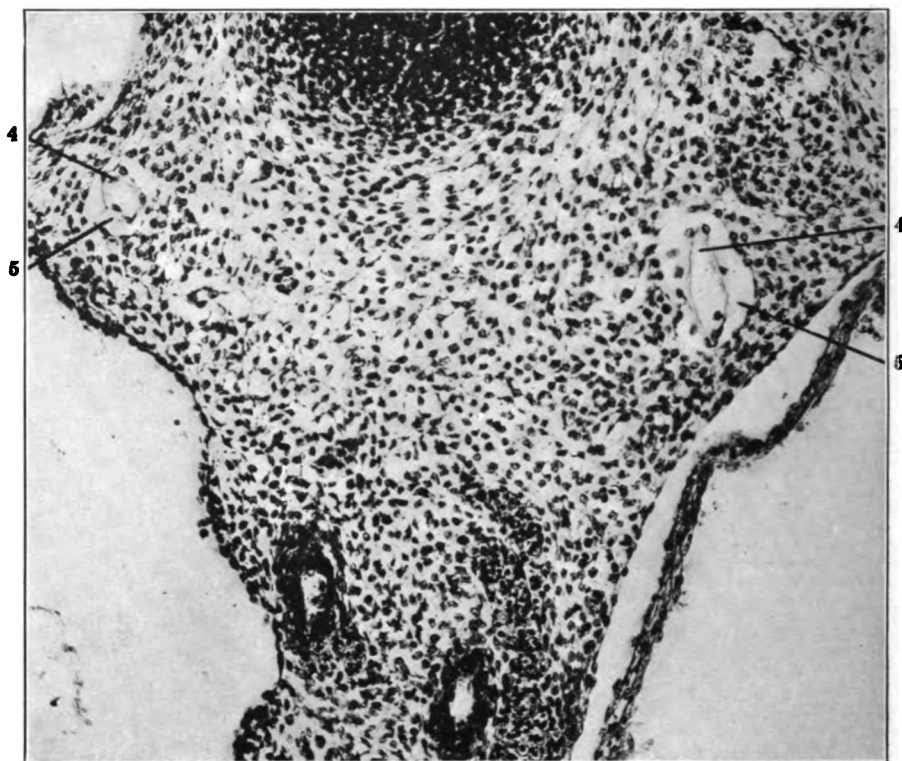


Fig. 4 Same, section 37.

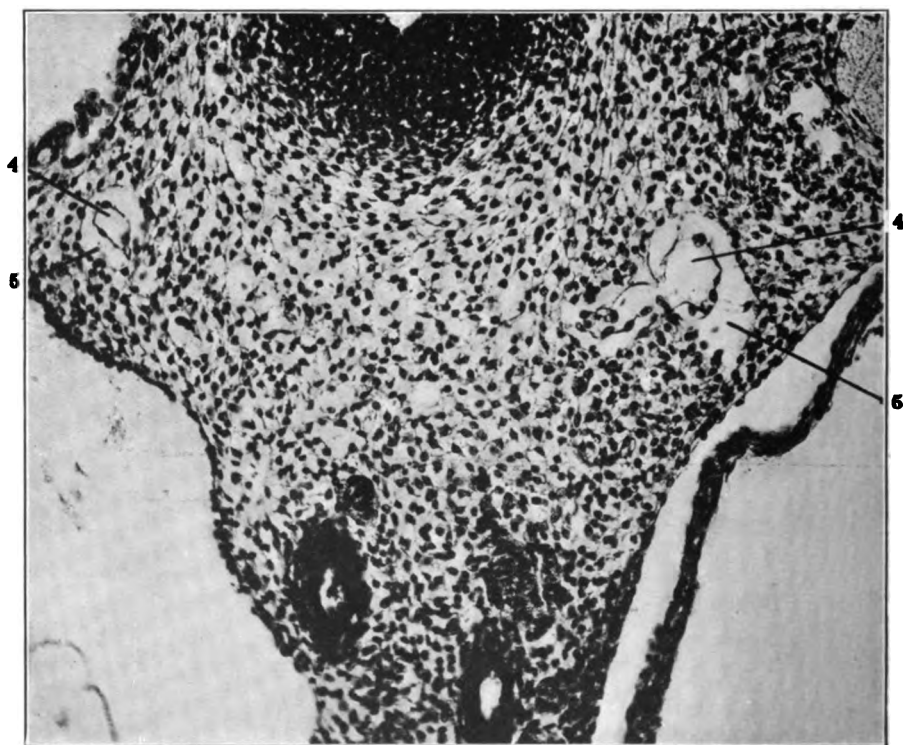


Fig. 5 Same, section 38.



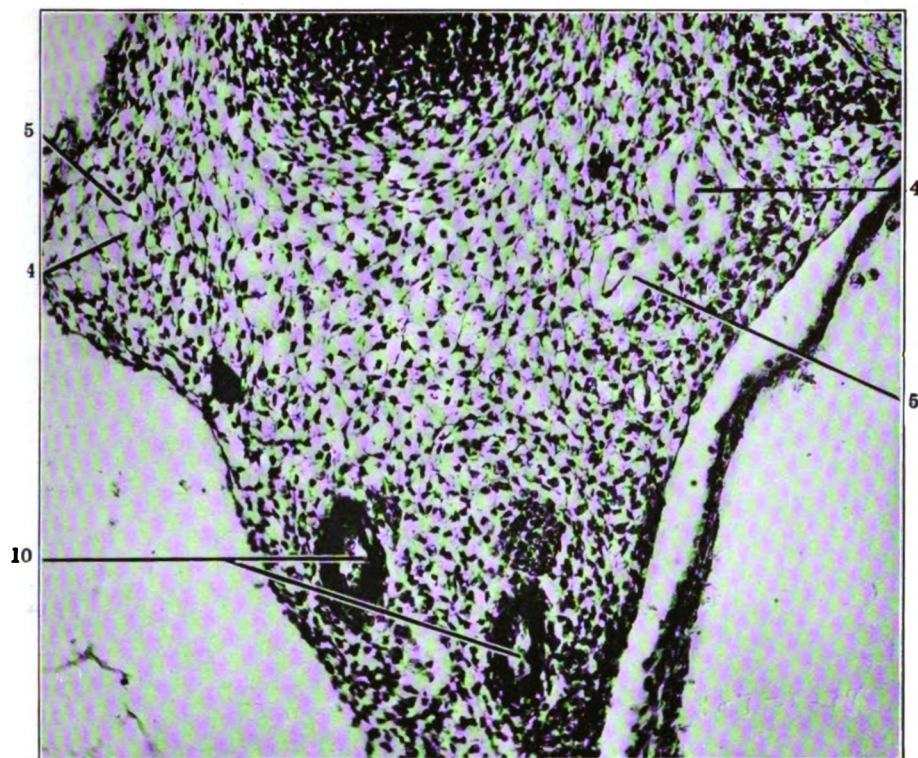


Fig. 6 Same, section 39.

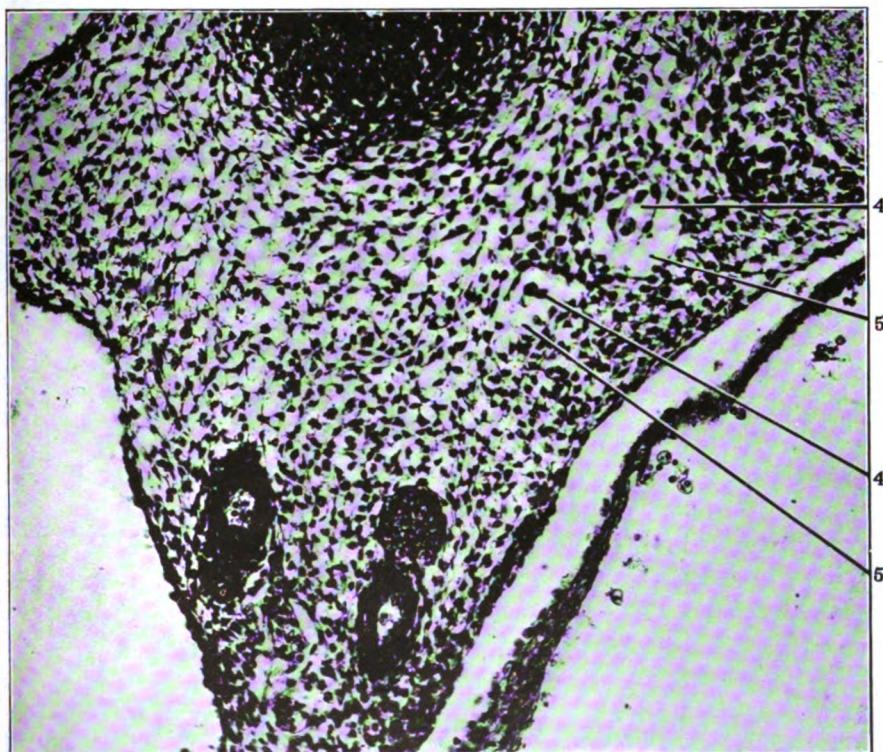


Fig. 7 Same, section 40.



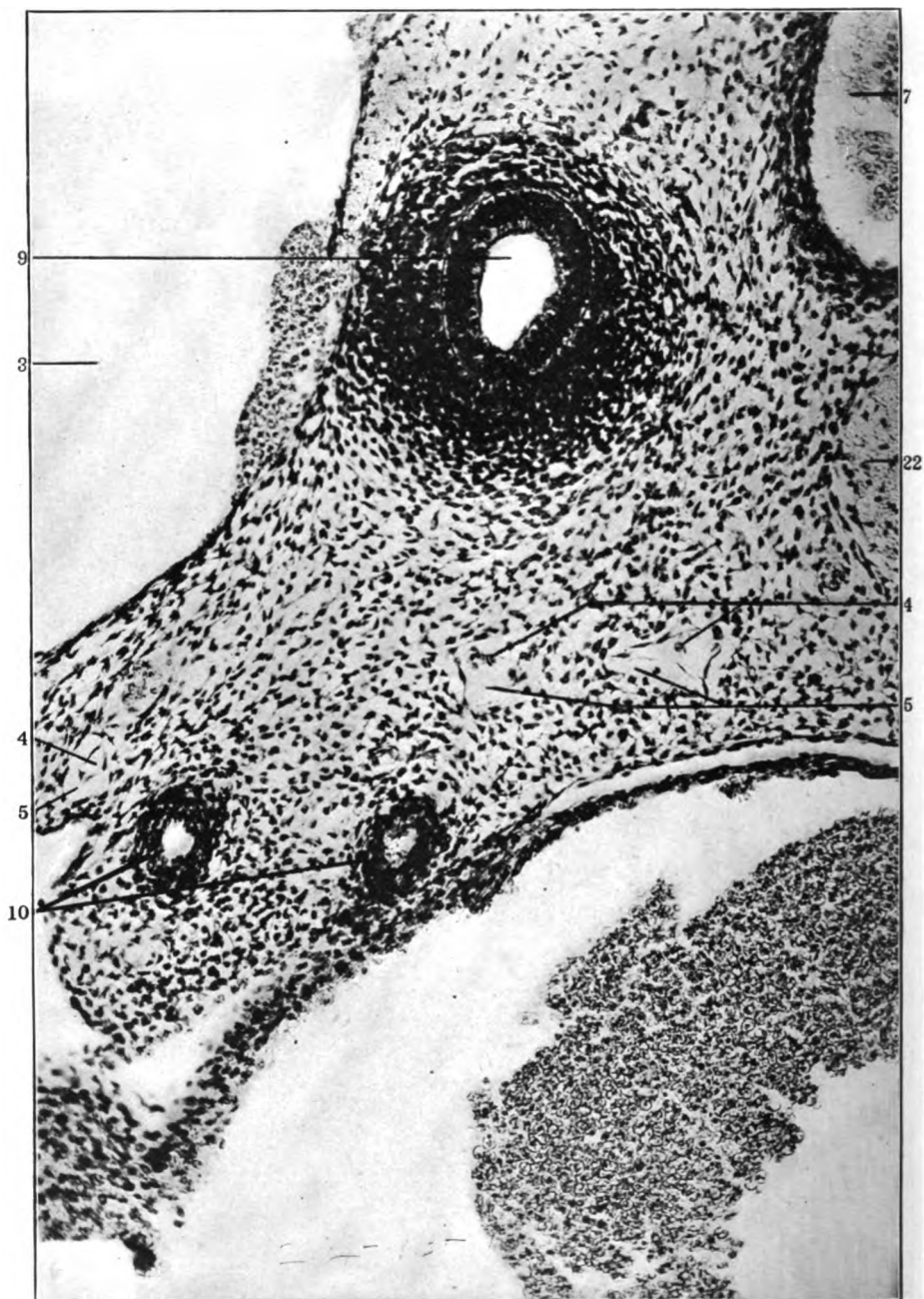
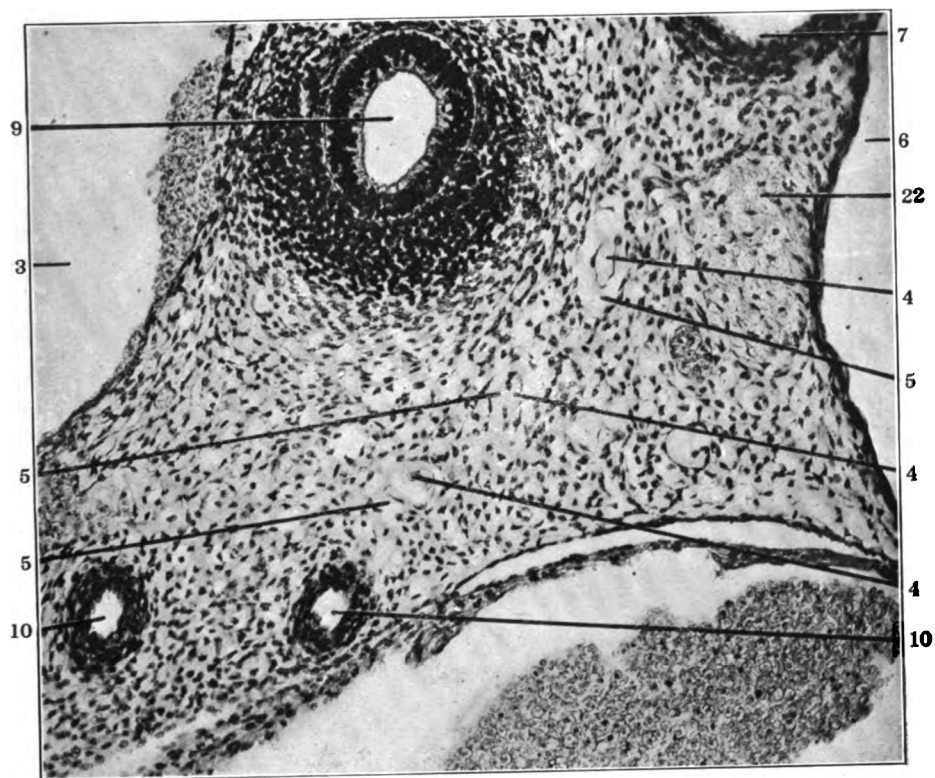
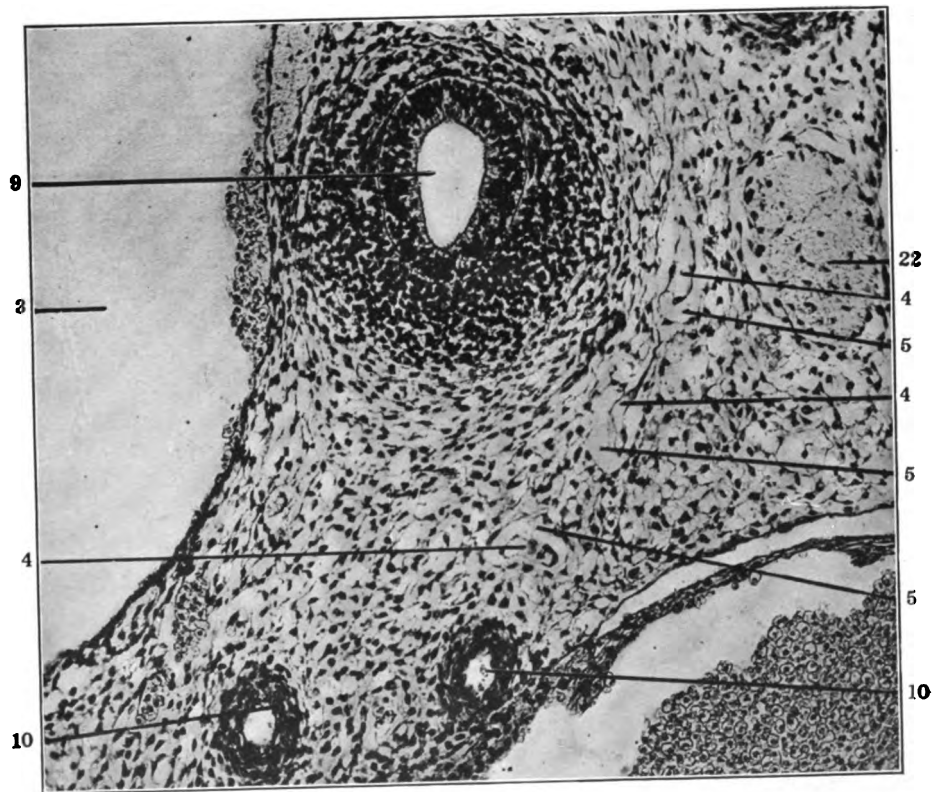


Fig. 8 Transverse section of anterior thoracic region of a 14 mm. cat embryo (series 214, slide 13, section 13,  $\times 225$ ).

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**Fig. 9** Same, section 15.



**Fig. 10** Same, section 16.

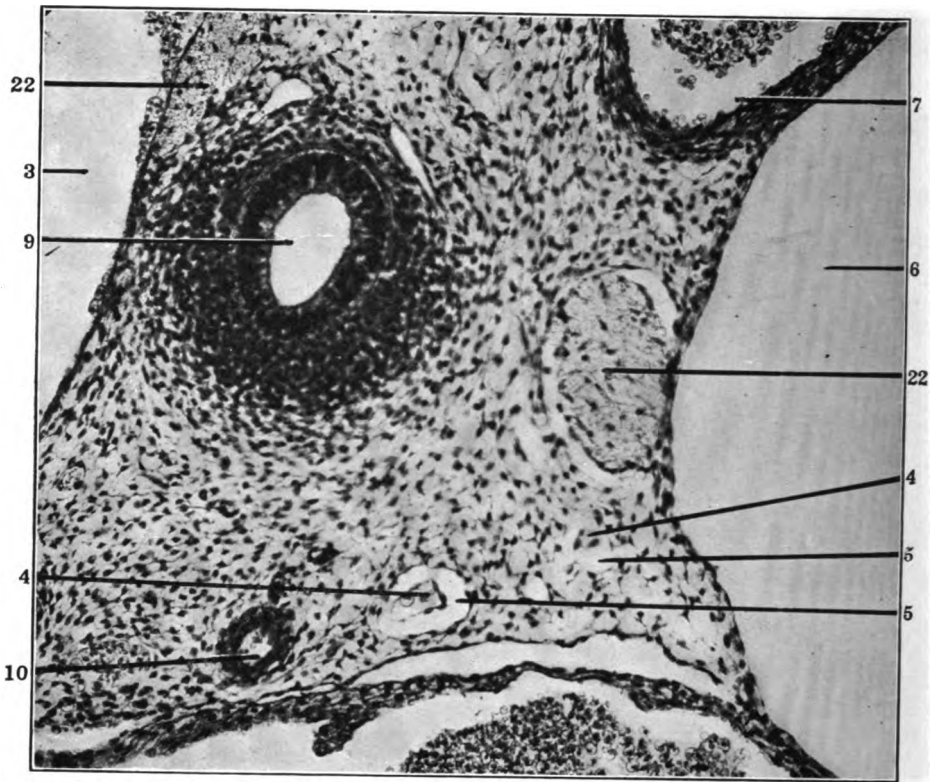


Fig 11. Same, section 21.

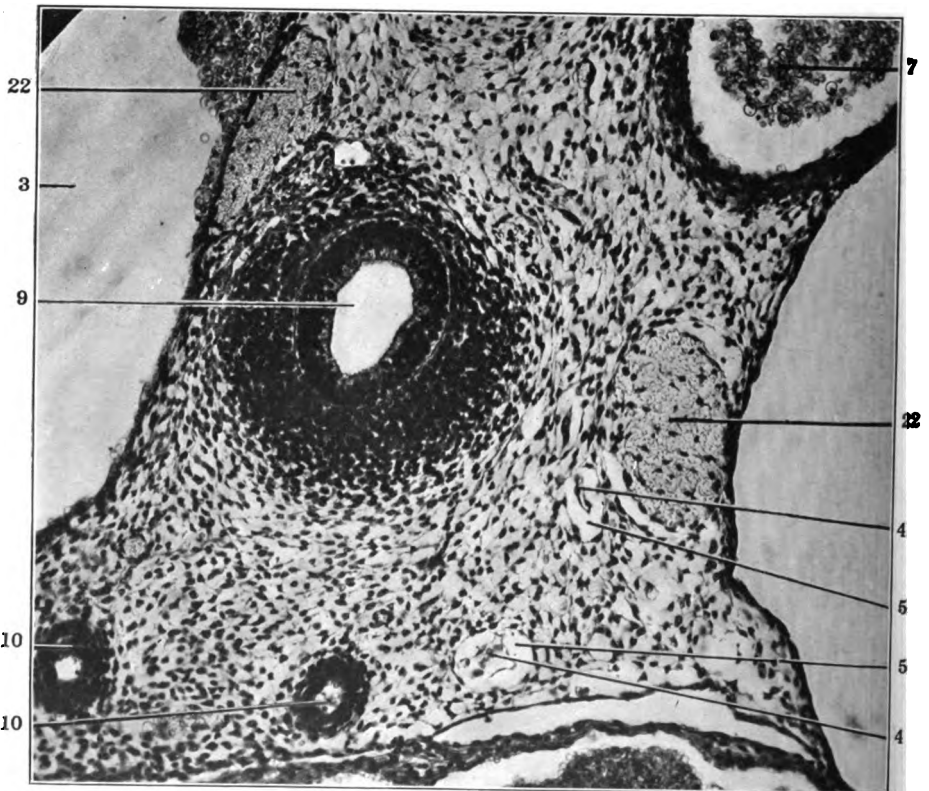


Fig. 12 Same, section 22.

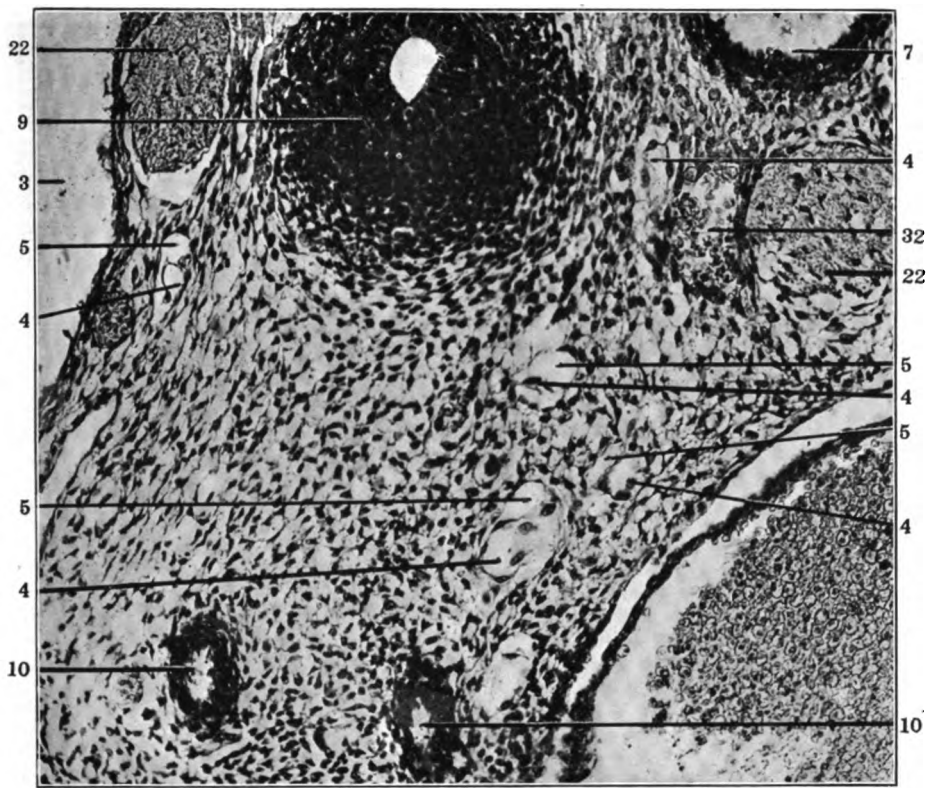


Fig. 13 Transverse section of anterior thoracic region of a 14 mm. cat embryo, (series 212, slide 10, section 5,  $\times 225$ ).

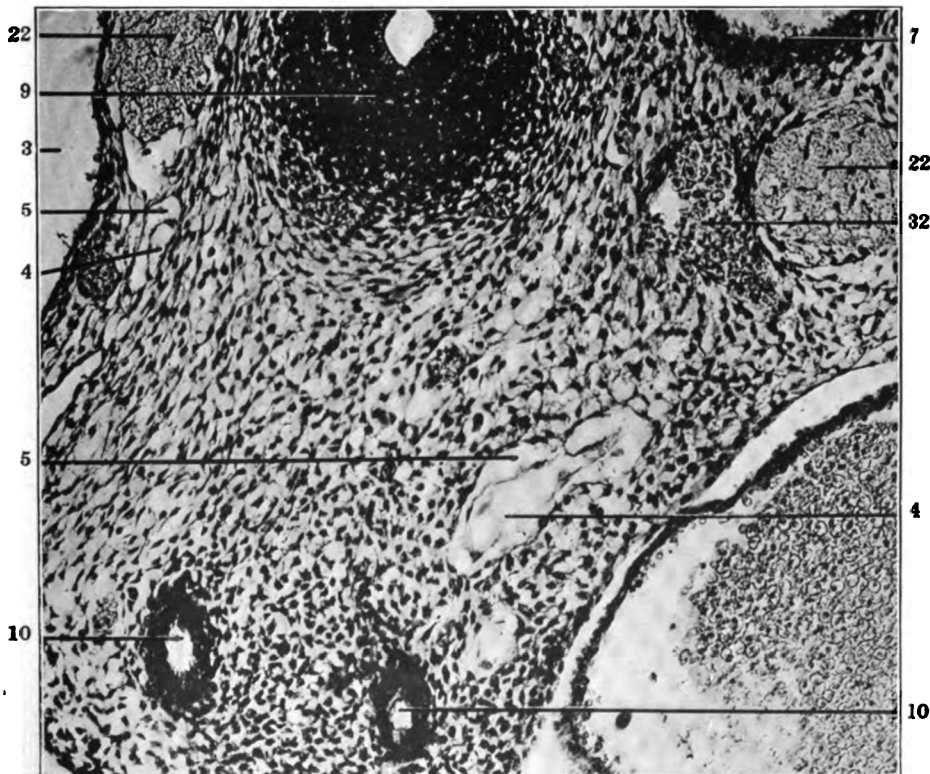


Fig 14 Same, section 6.



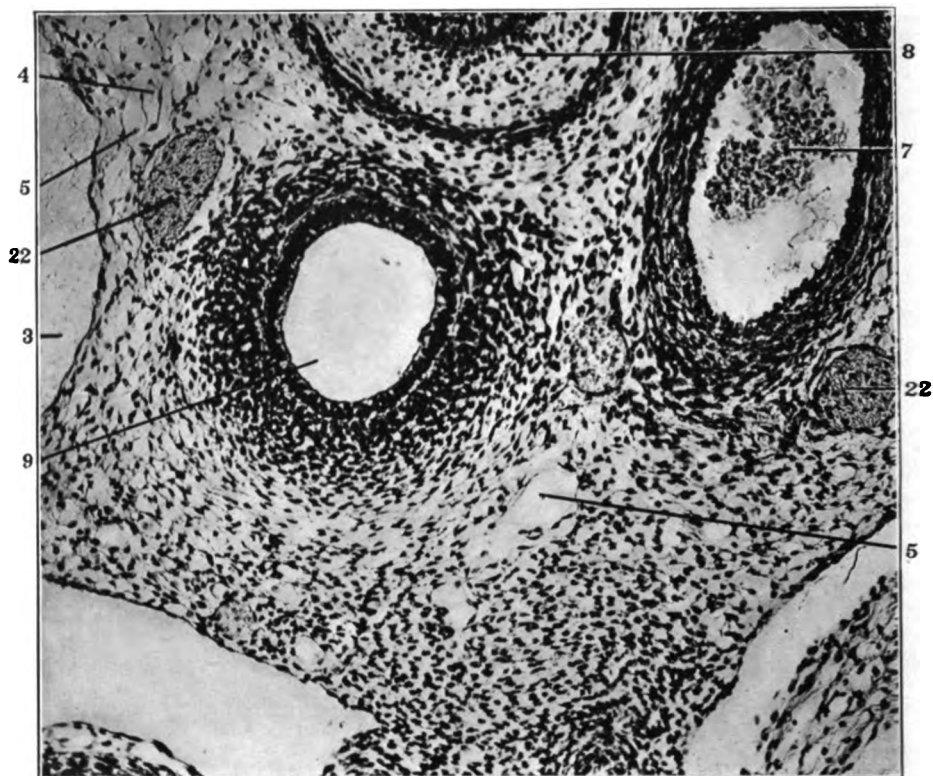


Fig. 15 Transverse section of anterior thoracic region of a 15 mm. cat embryo, (series 216, slide 10, section 32,  $\times 225$ ).

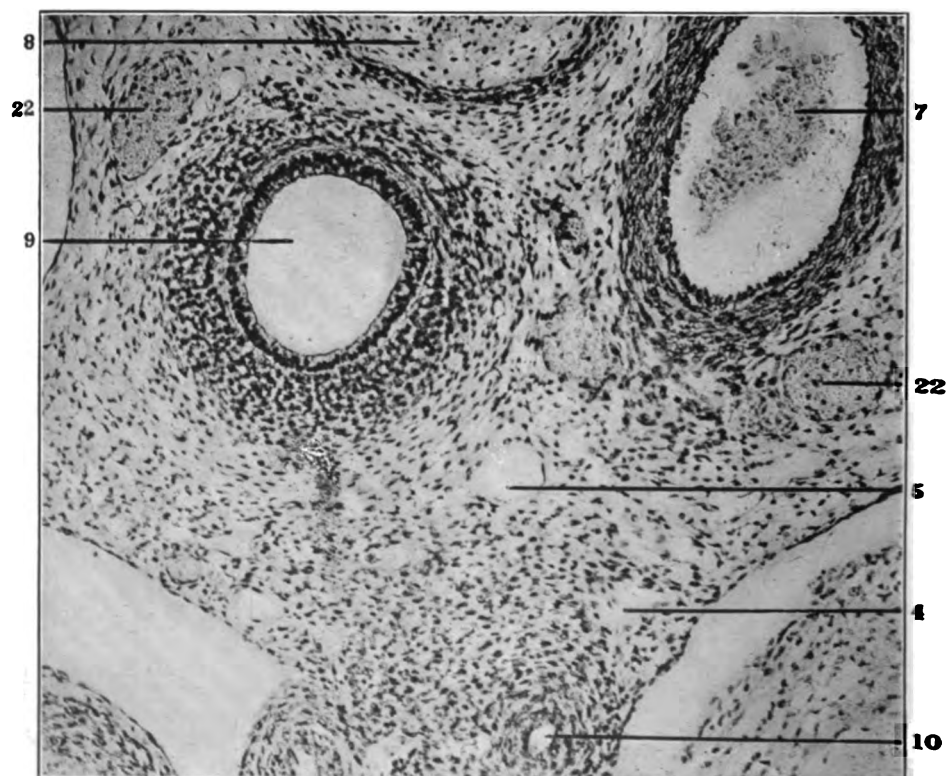


Fig. 16 Same, section 33.

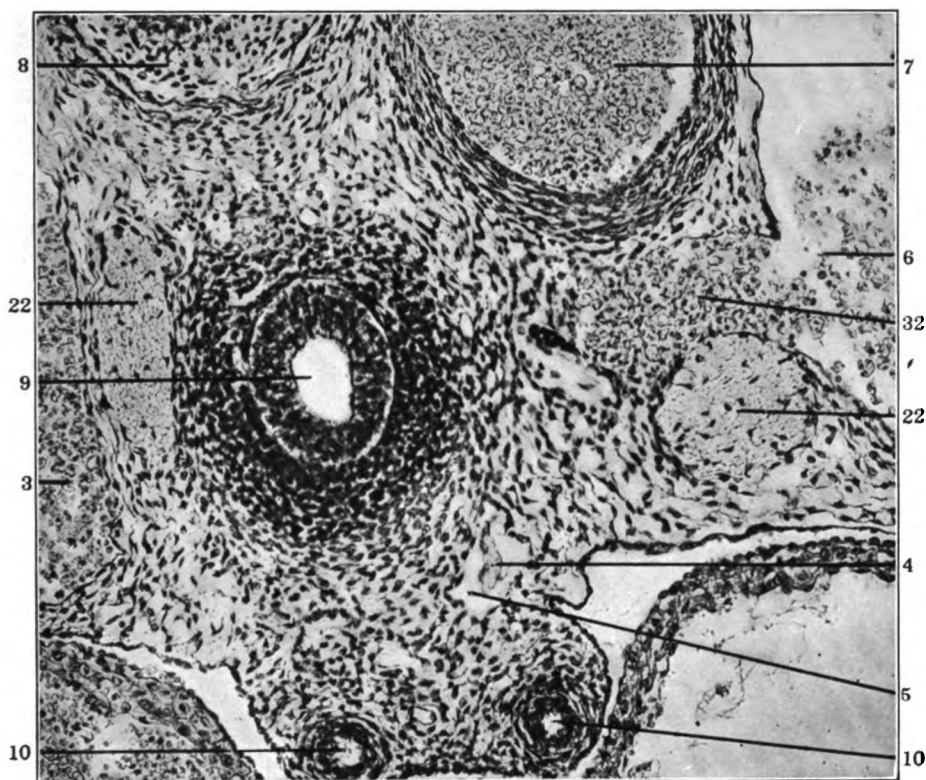


Fig. 17 Transverse section of anterior thoracic region of a 15.5 mm. cat embryo (series 215, slide 14, section 13,  $\times 225$ ).

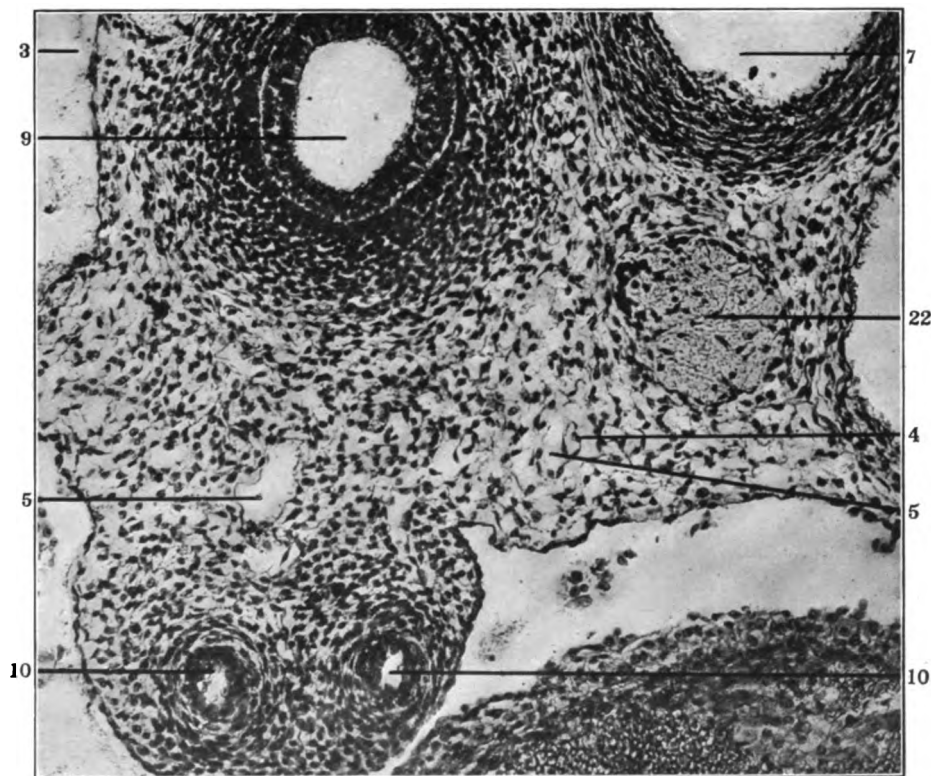


Fig. 18 Transverse section of anterior thoracic region of a 16 mm. cat embryo (series 220, slide 12, section 25,  $\times 225$ ).

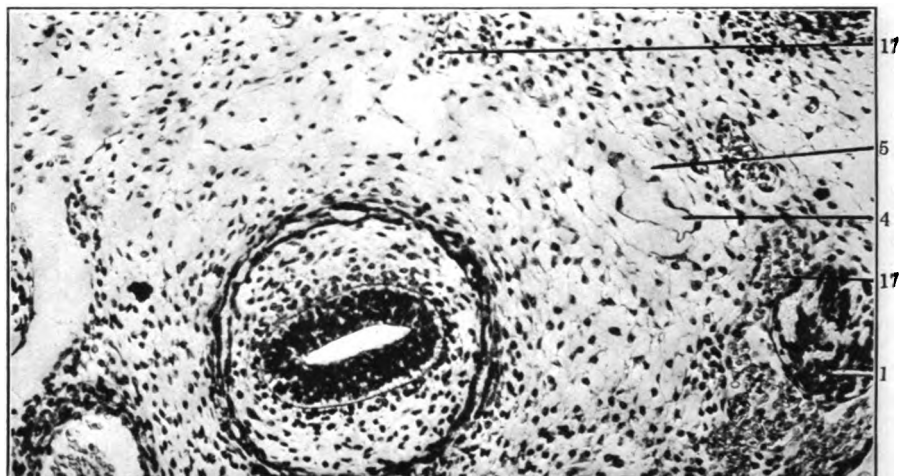


Fig. 19 Transverse section of anterior thoracic prevertebral area of a 14 mm. cat embryo, (series 214, slide 9, section 23,  $\times 225$ ).

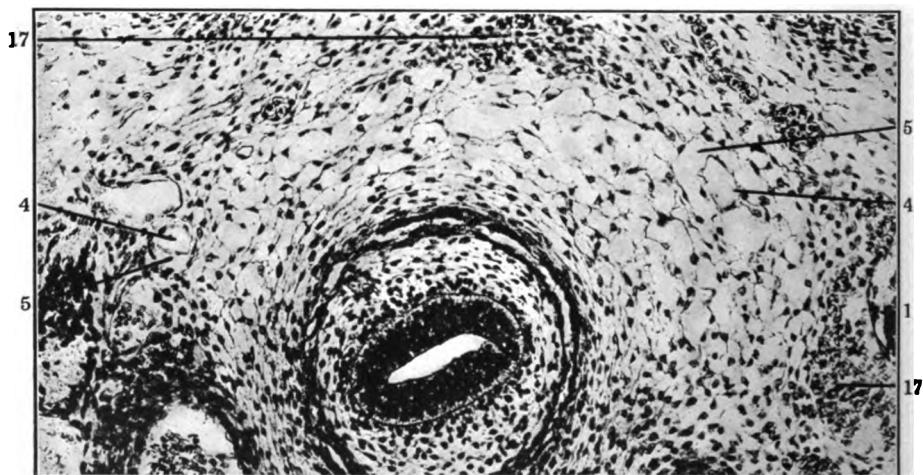


Fig. 20 Same, section 26.

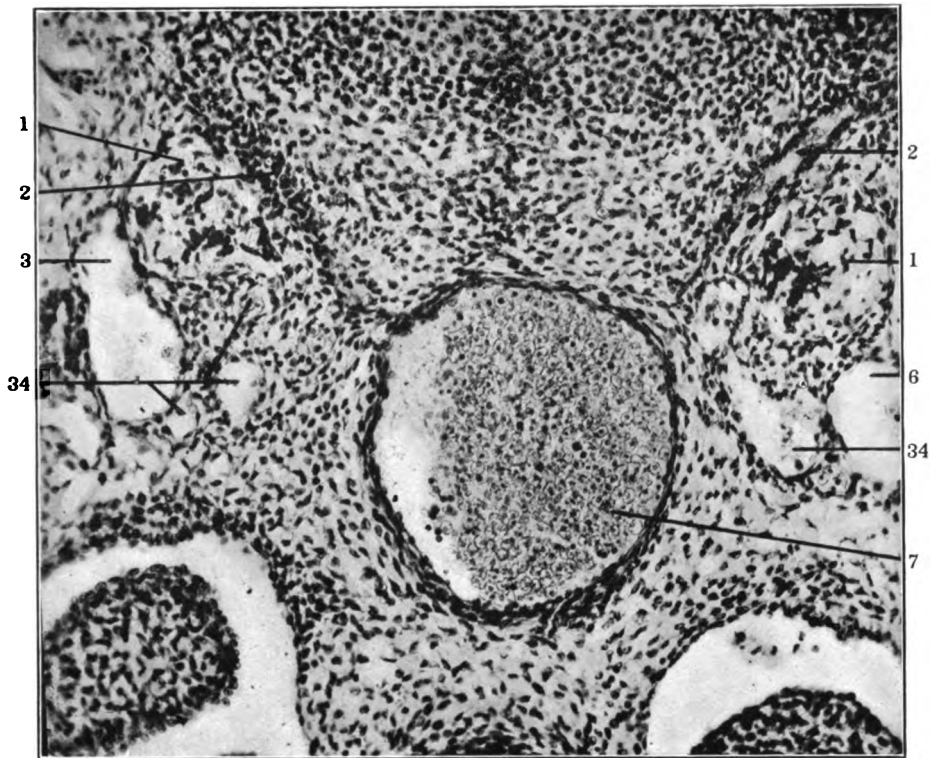


Fig. 21 Transverse section of middle thoracic region of a 11 mm. cat embryo, (series 213, slide 11, section 29,  $\times 225$ ).

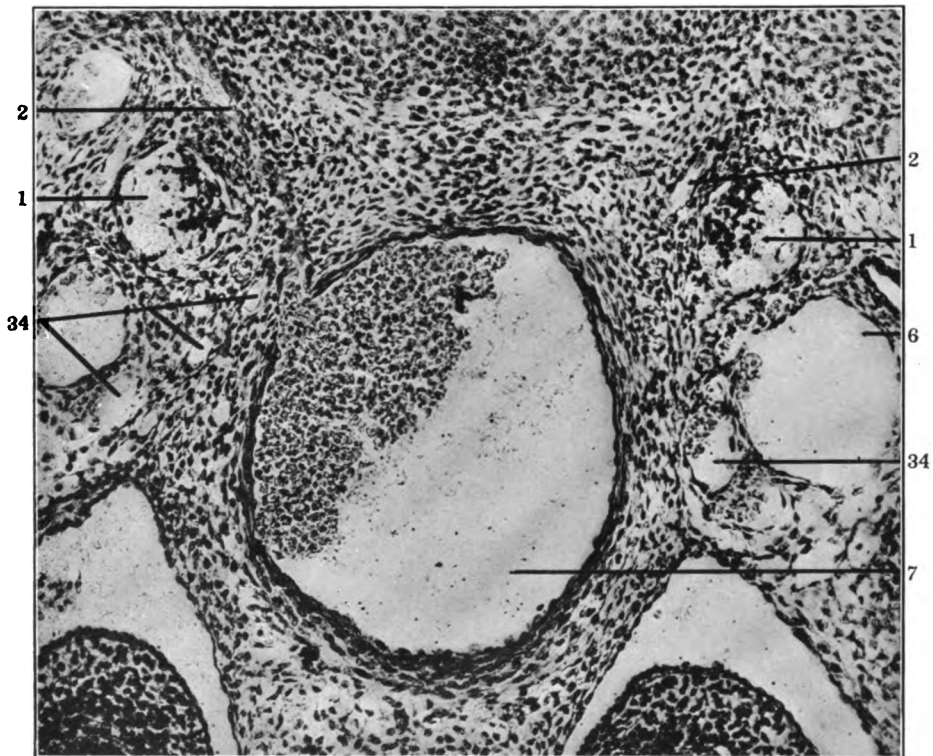


Fig. 22 Transverse section of middle thoracic region of a 12 mm. cat embryo, (series 217, slide 11, section 27,  $\times 225$ ).





Fig. 23 Transverse section of middle thoracic region of a 14 mm. cat embryo, (series 34, Princeton University Embryological Collection, slide 31, section 18,  $\times 225$ ).



Fig. 24 Same, section 19.



Fig. 25 Same, slide 28, section 19.



Fig. 26 Same, section 20.

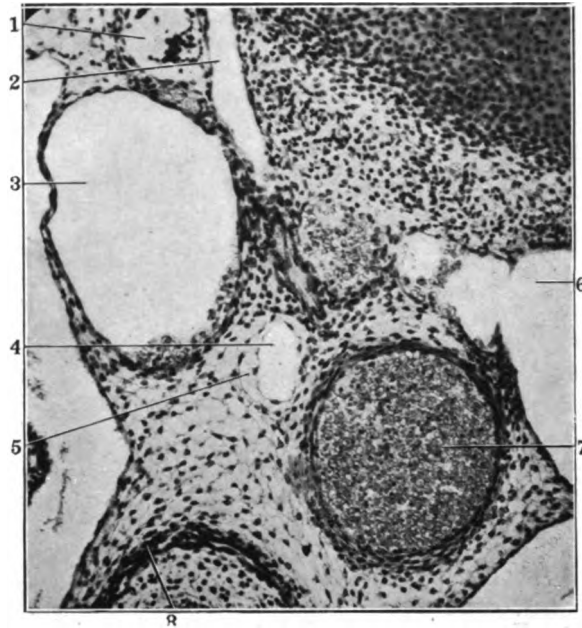


Fig. 27 Transverse section of middle thoracic region of a 14 mm. cat embryo (series 214, slide 15, section 10,  $\times 225$ ).



Fig. 28 Same, section 11.



Fig. 29 Same, slide 14, section 16.



Fig. 30 Same, section 17.

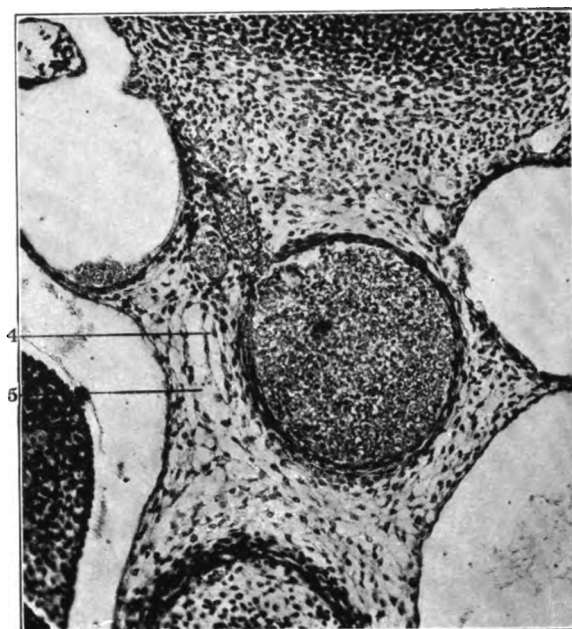


Fig. 31 Same, slide 15, section 27.



Fig. 32 Same, section 28.

# A NOTE ON POST-CARDINAL OMPHALO-MESENTERIC COMMUNICATIONS IN THE ADULT MAMMAL

ALFRED JEROME BROWN

*From the Anatomical Laboratory of Columbia University*

## THREE PLATES

Communications between the portal and systemic venous systems have been noted in many adult forms but have never been accounted for upon an embryological basis.

Krause<sup>1</sup> mentions several communications between the portal and systemic systems in the adult human, the only one of interest in connection with the subject of this paper being the branch which he describes as arising from the plexus around either the small or large intestine and emptying either into the inferior vena cava or the renal vein.

In the cynocephalous primates there is, in the adult, a capillary plexus between the two leaves of the mesentery which connects the radicles of the mesenteric veins with the sex veins which are the representatives of the embryonic post-cardinals.

In the bird, there is constantly present in the adult form, a coccygeo-mesenteric vein, which arises at the point of bifurcation of the caudal veins, runs parallel to the rectum, from which it receives tributaries, and empties into the portal vein (Parker and Haswell).<sup>2</sup>

In an adult cat, a hitherto undescribed connection between the portal and systemic systems of veins was found. In this animal at the point of junction of the sex vein with the inferior vena cava (which, in the post-renal segment, was formed by the embryonic left post-cardinal vein as shown by its dorsal relation to

<sup>1</sup> Henle: *Anatomie des Menschen*, Band 3', p. 394.

<sup>2</sup> Parker and Haswell: *Textbook of Zoölogy*, vol. 2, p. 374.

the left ureter), a large trunk was given off which passed forward, ventral to the left ureter, and to the right between the leaves of the mesentery and joined with the inferior mesenteric vein. This junction between the cross communicating vessel and the inferior mesenteric vein resulted in a large vein which ran cephalad through the mesentery, receiving tributaries from around the gut in its course, followed the normal course of the inferior mesenteric vein, and finally emptied into the superior mesenteric vein (see plate 1).

The occurrence of these communications between the portal and systemic systems in the adult mammal and their close correspondence to the normal coccygeo-mesenteric vein of the bird, naturally suggested that the communication was a persistence of an embryonic channel between the omphalo-mesenteric and cardinal systems which was normal at some period of growth.

In the cat, embryo of 10.4 mm. (series 81), immediately below the sub-cardinal cross anastomosis there is a plexus of small veins which extends across the root of the mesentery connecting the sub-cardinal veins of the two sides, but there are no distinct vessels which can be traced into the mesentery to connect with the plexus around the gut, and all of the veins draining the gut appear to run cephalad through the mesentery to empty into the omphalo-mesenteric veins.

In an earlier stage, however, cat of 6.5 mm. (series 131), a connection between the two systems can be clearly established. At this stage there is no distinct sub-cardinal system of veins, but this line is represented by a series of vascular spaces surrounding the individual mesonephric tubules which lie ventral to the post-cardinal veins which are of equal size.

At the level of the origin of the supra-renal artery from the aorta a small vein passes from the plexus around the gut dorsad through the mesentery, curves laterad at its root and finally empties into the venous spaces around the mesonephric tubules; further caudad another small vein pursues an identical course. These vessels are alike on the two sides and make a double tier-like connection between the omphalo-mesenteric and future sub-cardinal systems.

At the level of the junction of the middle and lower thirds of the mesonephros, the plexus of veins in the mesentery is represented by a single large trunk which passes dorsad from the gut to the root of the mesentery and there bifurcates. From this bifurcation a vessel passes on either side, laterad and dorsad between the aorta to the mesial and the mesonephros to the lateral side and taps the post-cardinal vein on its ventro mesial surface (see plates 2 and 3). This vessel is quite large and undoubtedly represents the embryonic channel of which the communications noted in the adult animals are remnants.

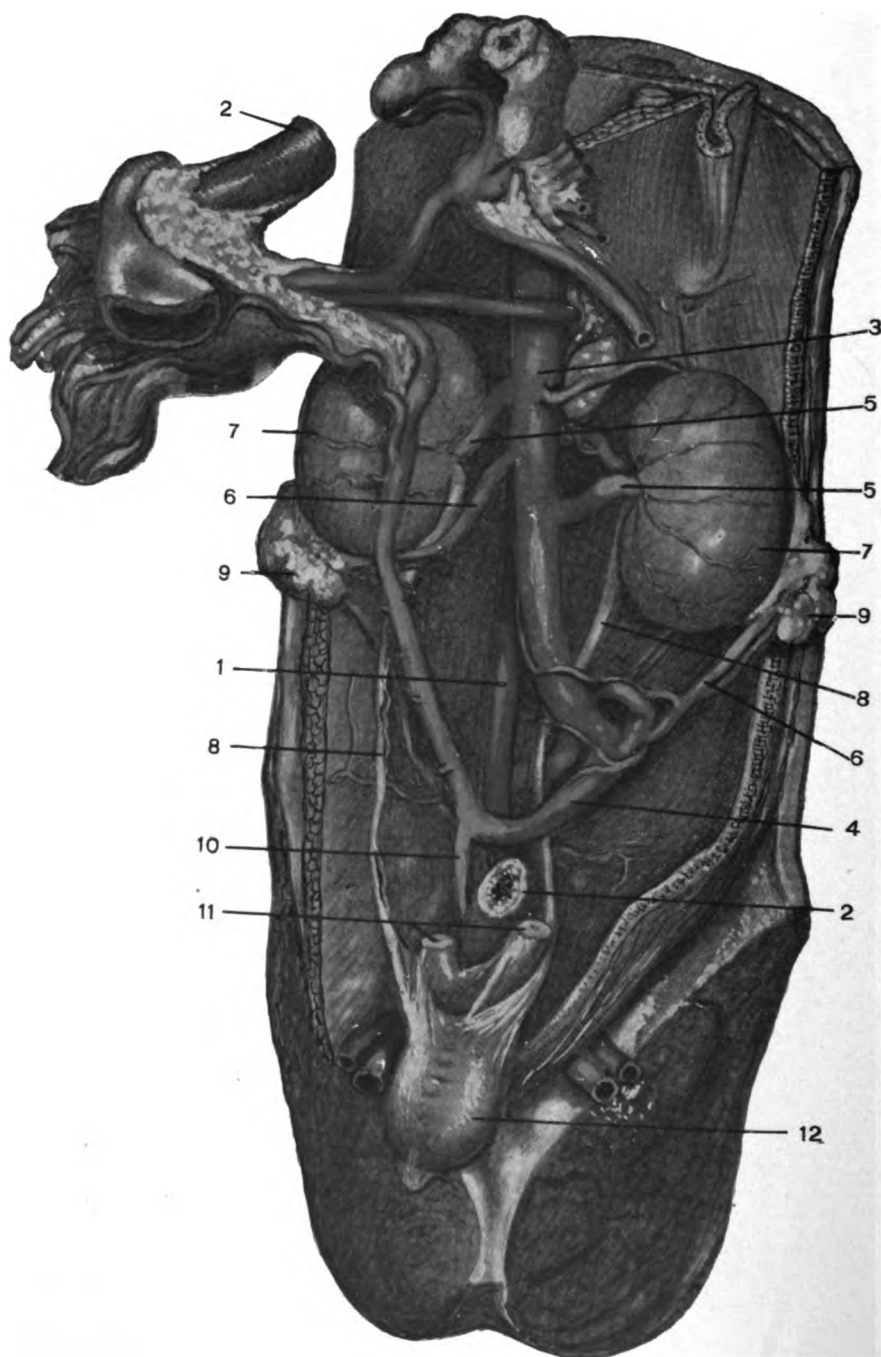
Thus there may be in the adult mammal a well defined communication between the portal and post-caval systems in addition to the usual communication through the capillaries of the liver, and this communication is distinctly analogous to the coccygo-mesenteric vein of the adult bird type and is a vestige of a communication between the omphalo-mesenteric and post-cardinal systems which exists in the mammalian embryo at an early stage of its development.

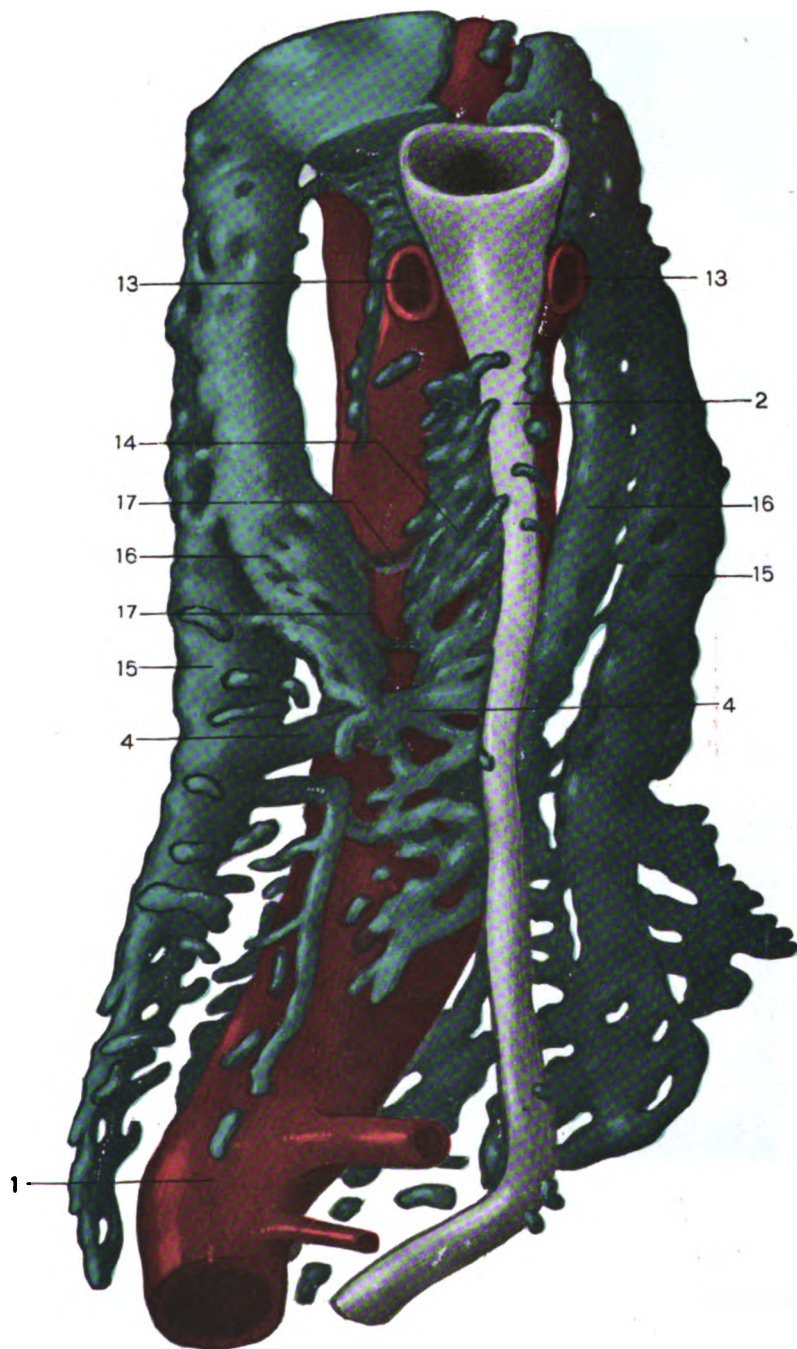


#### EXPLANATION OF PLATES

- |                             |                                    |
|-----------------------------|------------------------------------|
| 1 Aorta                     | 12 Bladder                         |
| 2 Intestine                 | 13 Omphalo-mesenteric artery       |
| 3 Inferior vena cava        | 14 Mesenteric venous plexus        |
| 4 Cross anastomatic trunk   | 15 Post-cardinal vein              |
| 5 Renal vein                | 16 Rudimentary subcardinal line    |
| 6 Sex vein                  | 17 Communication between 14 and 16 |
| 7 Kidney                    | 18 Wolffian duct                   |
| 8 Ureter                    | 19 Mesonephros                     |
| 9 Ovary                     | 20 Umbilical vein                  |
| 10 Inferior mesenteric vein | 21 Body wall                       |
| 11 Uterus                   |                                    |

## PLATES







# SOME FEATURES OF THE HISTOGENESIS OF THE THYREOID GLAND IN THE PIG

ROBERT ORTON MOODY

*From the Hearst Anatomical Laboratory of the University of California*

FOURTEEN FIGURES

That the thyreoid gland of pig has its origin in a median and two lateral elements which unite early in embryonic life to form a one lobed gland, lying ventrad of the trachea, was definitely determined by Born and confirmed by other investigators. But concerning certain features of its histogenesis, different views have been expressed. The development of the connective tissue framework, the processes and relation of follicle and colloid formation and some other disputed points are the subjects of this investigation.

Wölfler, one of the earlier investigators, is quoted by Lustig as follows:

"The epithelial vesicles are formed from masses of round or elongated cells having large, round nuclei surrounded by very little protoplasm. Towards the end of the foetal period and after birth the peripheral elements of these groups of cells dispose themselves in a circle and assume a cubical form, while the central elements become at first granular, then degenerate and disappear in the pale granular mass that fills the lumen of the vesicle thus formed, which is lined with epithelium." Lustig then adds "concerning the form, size and general characteristics of the epithelial masses and their transformation, my observations agree entirely with those of Wölfler."

Hertwig describes the formation of the vesicles as follows:

"The cords acquire a narrow lumen around which the cylindrical

cells are regularly arranged. Then there are formed on the cords at short intervals enlargements, which are separated by slight constrictions. By the deepening of these constrictions the whole network is finally subdivided into numerous, small, hollow, epithelial vesicles or follicles, which are separated from one another by highly vesicular embryonic tissue. Subsequently the follicles increase in size, especially in the case of man. This is due to the secretion by the epithelial cells of a considerable quantity of colloid, which is poured into the cavity of the follicles."

Soulié and Verdun in their study of the development of the thyroid in rabbits and moles, referring to a rabbit embryo of 15 mm. say: "The cords which constitute the median thyroid no longer present a uniform caliber throughout their entire length; at intervals they show swellings which are hollow ampullae lined with cubical epithelium. This is the first appearance of the follicles of the gland."

Tournaux and Verdun, describing the thyroid in a human embryo of 32.4 mm. say: "The cell cords have not a regularly cylindrical form but carry throughout their length spherical or ovoid enlargements, in which there are central cavities. The cords average 30-40 microns in diameter, increasing to 80 at the level of the dilatations, which are formed of small polyhedral cells heaped on each other around the central excavation. In many places the wall of the vesicle appears thickened in the form of a bud, which gives the external surface a varicose appearance."

Thus it is seen that Wölfler and Lustig found in the pig and some other animals that the formation of the follicle and the colloid are synchronous, late in foetal life, both are formed by the degeneration of the central portion of masses of cells. Soulié, Tournaux and Verdun, however, find that in man, rabbit and mole, follicles appear early in foetal life, formed from swellings on, or enlargements of the primitive cell columns; and that the formation of colloid takes place at a later period. Hertwig offers another slightly different view: that a lumen first appears in the cords, upon which alternate enlargements and constrictions occur later to form the follicles.

## TECHNIQUE

Embryo pigs in the earlier stages, 5 to 35 millimeters in length, were fixed in Zenker's fluid, cut in serial sections 5-10 microns thick and stained with Mallory's connective tissue stain, as modified by Sabin or with haematoxylin and congo red.

From older embryos, 40-280 millimeters in length, the glands were removed, fixed in Zenker's, or in van Gehuchten's fluid, and prepared as above for general study. For the further study of the connective tissue framework, two methods of digestion were used: Flint's method of piece digestion for the demonstration of the framework of organs and Hoehl's method of the digestion of thin sections on slides, every alternate section being kept without digestion for control, as suggested by Clark.

In Flint's method, which the author characterizes as "tedious at the best," the time element is most variable and uncontrollable. Of two sections equally thick, cut from the same gland, carried through all stages of digestion in the same containers, one digests in three or four weeks, while the other takes as many months. The one that digests more slowly usually appears brown after a few days, while the other retains its normal color and becomes more transparent. Both eventually yield satisfactory results. The process of fat extraction may be omitted with embryonic tissues, thereby shortening the time required for digestion by ten or twelve days. It is especially desirable to omit a second extraction in older tissues, not only on account of saving time, but also to avoid injury to the sections, which adhere closely to the walls of the paper box container, so that it is almost impossible to remove them without more or less destruction of the delicate tissues. The most satisfactory method of removing the pieces of gland from the paper box is to open the latter and immerse it in a dish containing digesting fluid, after which gentle shaking may free the tissue. The use of any other mechanical force usually results in some distortion or tearing.

To ensure success with this method certain precautions must be observed. All glassware, corks, etc., must be chemically clean as the presence of even a minute quantity of certain reagents



interferes with or entirely inhibits the digestive process. All fluids should be carefully filtered, for any small particle of foreign matter may become entangled in meshes of the digesting tissue and greatly interfere with the study of the framework. When changing the fluid it is not necessary nor advisable to remove all of it from the vessel containing the sections, but enough should be left in the dish to float them in order to avoid distortion and tearing of the tissues. If for staining or any other process the specimens are to be transferred from one dish to another, a spoon with a small bowl placed at right angles to the handle is desirable.

An excellent picture of the coarser framework of organs can be seen by the use of the stereoscopic microscope, long before digestion is complete. It is advantageous to study and draw the sections at this stage, because as digestion proceeds, in spite of every precaution, delicate tissues may become twisted or torn and the complete picture ruined. The specimen may be removed from the digestive fluid, washed in water, put in glycerine, studied, rewashed in water and replaced in the fluid to complete digestion. The transfer from water to glycerine and back to water should be made through several dilutions of increasing strength. After digestion is completed the structure of the framework may be more strongly brought out by staining the tissue with aniline blue. It is possible to use the oil immersion to advantage in studying the finer details of thick sections.

While using Hoehl's method of digesting sections on slides, it was found that with a slight addition to the technique sections 200 microns thick may be prepared. These sections are fastened to the slide in the following manner: after removing the paraffine in the usual way sections are placed in absolute alcohol for a few minutes and then put on the slide. A fine camel's hair brush dipped in thin celloidin is put at four equidistant points of the periphery of the section and from each point is drawn quickly toward the edge of the slide. The four celloidin bands thus made hold the section to the slide, not only during digestion but also through the subsequent processes of staining and mounting.

By this method it is possible to study the framework of embryonic organs in three dimensions with the various powers of the

monocular microscope, whereas in young embryos even the gross structure is so small that piece digestion and the stereoscopic microscope fail to reveal it.

After digestion, to avoid injury to the tissues, all fluids used in washing, staining and dehydrating must be put on the slide at the edge of the section drop by drop and allowed to spread slowly. Pieces of blotting paper used to absorb the fluids should never be placed on the tissues.

#### DESCRIPTIONS

##### *Pig embryo 5 mm. in length*

In 5 embryos of this length, the median element of the thyroid gland is a compact syncytium forming a bi-lobed elongated mass of irregular outline, lying in the mesodermal syncytium on the ventral and lateral walls of the aorta, at about the level of the second gill-arch. It is still attached to the ventral wall of the pharynx by a cord of cells forming a pedicle that varies from 30 to 75 microns in length. The entire length of the gland, including the pedicle, varies from 75 to 155 microns. The two lobes may lie in close contact, with only a thin layer of mesodermal syncytium between them or they may be separated throughout their whole length by a blood vessel as well as the syncytium.

The line of division between the two lobes corresponds with the median line of the body, so that the lobes lie one each side of this plane. This line commonly terminates at the caudal end of the pedicle, but may extend throughout its entire length to the ventral wall of the pharynx (fig. 1). This condition together with the fact that the lateral elements of the gland are paired, suggests that at this stage the thyroid of pig is a paired organ.

The median element as a whole, following closely the contour of the aorta, has the shape of a piece of gutter, concave dorsad, convex ventrad. The surface in contact with the wall of the aorta is smooth, but the convex surface is studded with cell masses, varying greatly in size and shape.

The parenchyma of the gland is a syncytium with large, round or oval nuclei, which in two embryos are evenly distributed in an abundant cytoplasm (fig. 2).

In the other three embryos, a differentiation has taken place into an outer layer of closely crowded, elongated, oval nuclei, radially arranged in a scanty protoplasm, and an inner area of smaller, rounder nuclei with abundant protoplasm (fig. 1).

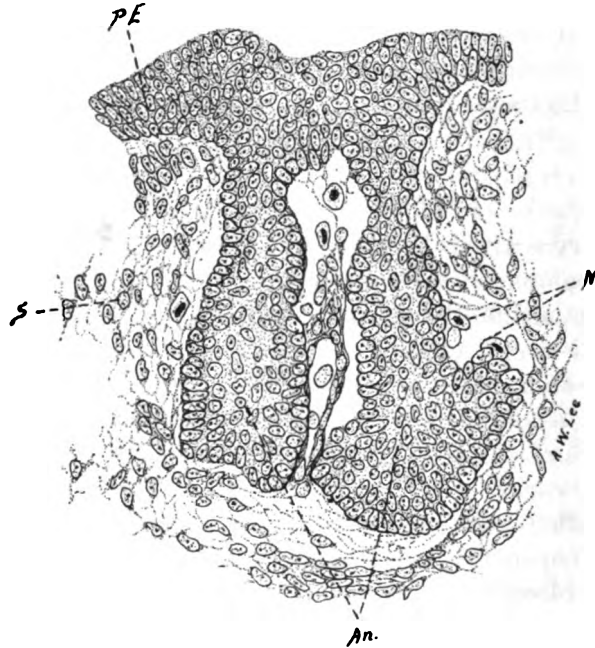


FIG. 1 Frontal section of thyroid of pig embryo 5 mm. in length. Magnified 175 diameters. An, median thyroid element. N, nucleated red blood corpuscles. PE, epithelium of pharynx. S, mesodermal syncytium.

This change when it has taken place, remains a characteristic feature until the median element is invaded by blood vessels in embryos 13–15 mm. in length. Neither size, shape nor staining properties distinguish the nuclei of the parenchyma from those of the surrounding mesoderm.

The mesodermal syncytium consists chiefly of round or oval nuclei and endoplasm. With Mallory's stain blue exoplasmic fibrils may be seen forming from the endoplasm, which has a pinkish tinge. Fibrils of exoplasm follow closely the contour of the gland forming a delicate investment, from which fibrils

may be seen passing into the parenchyma, not penetrating deeply, but surrounding one or two nuclei or passing between them. In addition to these delicate fibrils larger strands of exoplasm enter with blood vessels that pass through the gland. From the walls of these vessels or from these strands and occasionally from the

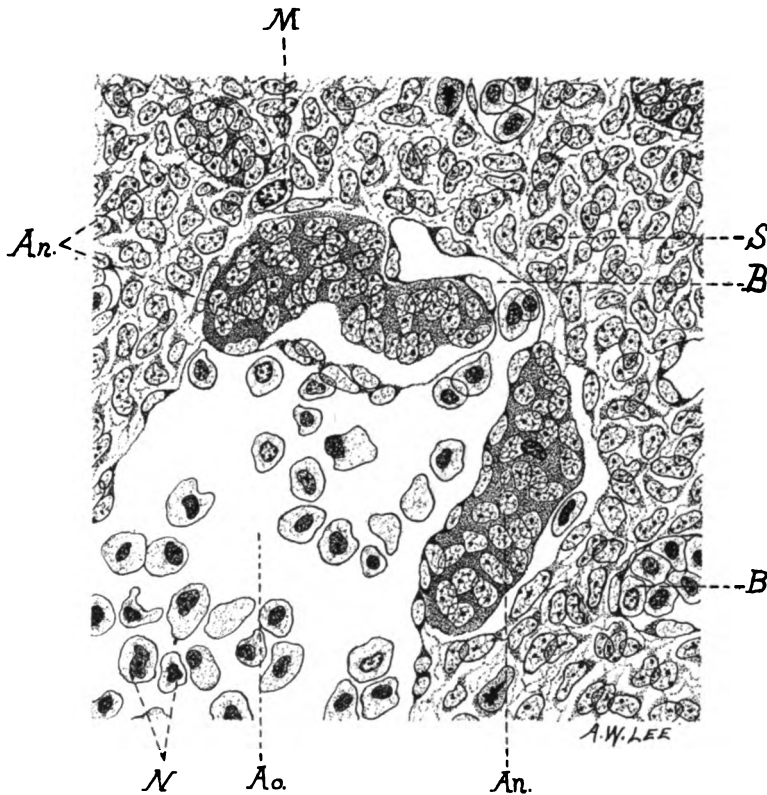


FIG. 2. Transection of thyroid of pig embryo 5 mm. in length. Magnified 555 diameters. An, median thyroid element. Ao, aorta. N, nucleated red blood corpuscles. S, mesodermal syncytium.

wall of the aorta fibrils of exoplasm extend into the parenchyma (fig. 3). These vessels arise from the aorta and pass directly through the median element without giving any branches to the gland.

*Pig embryo 6.5 mm. in length*

The median thyreoid elements of two embryos are still connected by a pedicle to the wall of the pharynx, but only in one of

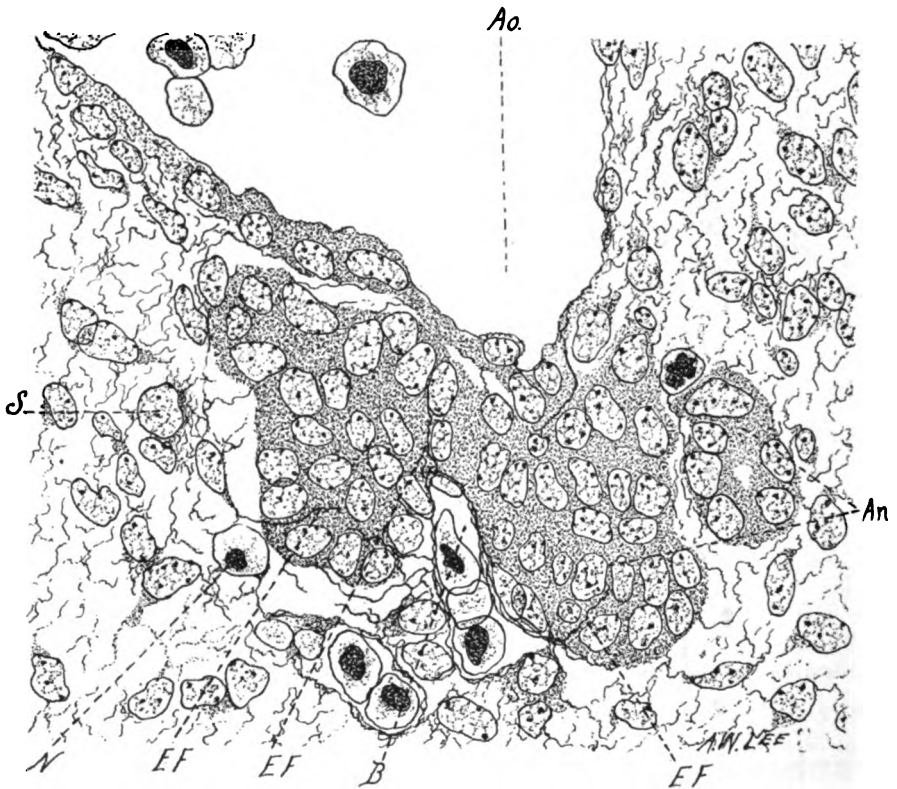


FIG. 3 Transection of thyreoid of pig embryo 5 mm. in length. Magnified 435 diameters. An, median thyreoid element. Ao, aorta. B, blood vessel. N, nucleated blood corpuscle. S, mesodermal syncytium. EF, exoplasmic fibrils.

them is it definitely bi-lobed. In the other it is extremely irregular in shape, being much cut up by the blood vessel winding through it. A branch from the aorta passes through the bi-lobed element, but this is the last stage prior to the general vascularization of this

element in which blood vessels are found within the gland. Increase in size is the only noticeable difference between the gland in these and in earlier embryos.

*Pig embryo 7 mm. in length*

Born describes the median element of the thyreoid of embryo pigs at this age as follows: "Aus einer kleinen Vertiefung zieht ein Epethelialstrang ventralwärts in der Länge von 0.1 mm. der sich zu einer von hinten her löffelartig ausgehöhlten Epithelmasse verbreitert. Die ausgehölte Mitte derselben ist sehr dünn so dass es oft den Anschein hat, als theile sich der Epithelstrang in zwei bogenförmig divergirende Aeste. Im Innern der seitlichen Enden waren Lumen erkennbar." This description indicates that the median element is bi-lobed in appearance only, but this investigation shows that the division into two lobes is real and definite, in this as in younger and older embryos. It also shows that no lumen such as Born describes is present in the median element at this or any other stage. It is true, however, that the pedicle has at this time separated from the wall of the pharynx.

*Pig embryo 10 mm. in length*

The changes that take place in the median element and the surrounding mesodermal syncytium during the development of the embryo from 7 to 10 mm. in length are chiefly those of rapid growth. At 10 mm. the cytoplasm is relatively less abundant and the nuclei more so than in earlier stages and many of the nuclei in both syncytia are in some phase of karyokinesis. There are around the periphery of the median element blood vessels that do not penetrate the parenchyma.

*Pig embryo 12-15 mm. in length*

At 12 mm. begins the invasion of the median element by blood vessels. Sometimes the direct connection is seen between blood

vessels without and within the gland, but frequently none was found between these extra-parenchymal vessels and spaces within, which contain nuclei of mesodermal origin and fibrils of exoplasm and appear to be blood vessels (fig. 4).

This invasion proceeds rapidly until embryos are 15 mm. in length, when the bi-lobed condition and differentiation of the

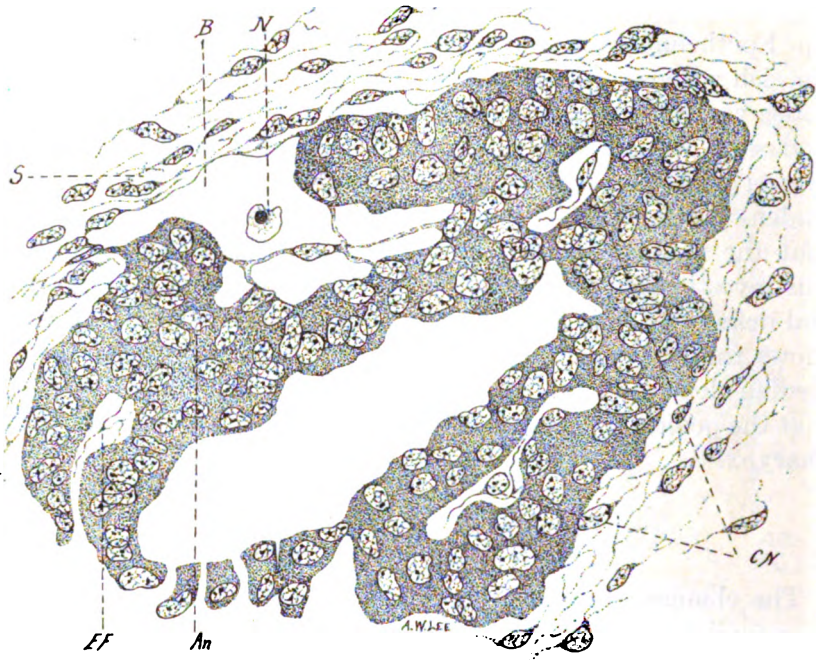


FIG. 4 Transection of thyroid of pig embryo 13 mm. in length. Magnified 555 diameters. An, median thyroid element. B, blood vessel. CN, mesodermal nuclei. EF, exoplasmic fibrils. N, nucleated blood corpuscles. S, mesodermal syncytium.

parenchymal nuclei into a distinct central and peripheral area no longer exists, but the nuclei are similar in shape and uniformly distributed throughout the parenchymal syncytium. The parenchyma is cut into many islands of various shapes and sizes by the blood vessels as is pictured by Born.

The lateral elements of the thyroid, which arise from the ventral ends of the fourth gill arch, are now flask-shaped and still

attached to the arch by a constricted neck, which as it has no lumen may be called a pedicle. These elements are formed of one or more layers of nuclei in a syncytial protoplasm lying in the mesodermal syncytium and surrounding a central cavity. Arising from this syncytium and continuous with it, fibrils of exoplasm

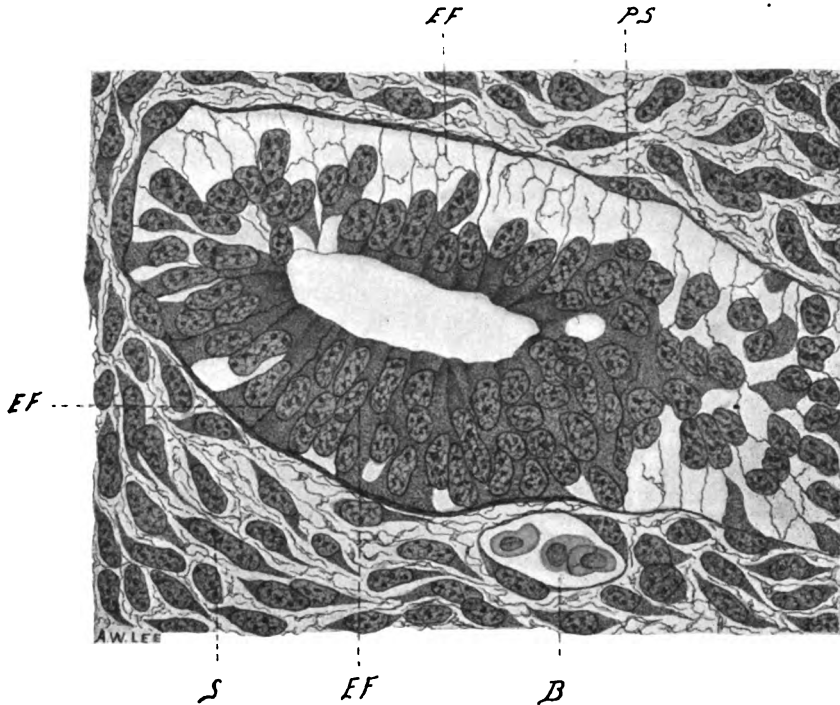


FIG. 5 Transection of the lateral element of the thyroid of pig embryo 15 mm. in length. Magnified 555 diameters. B, blood vessel. EF, exoplasmic fibrils. PS, parenchymal syncytium.

pass centrad, forming an intra-parenchymal exoplasmic framework.

Holmgren has described an intercellular connective tissue framework supporting the epithelial cells of the mucous membrane of the oesophagus in *Hirudo medicinalis* and *Proteus anguineus*.

The study of these early embryos shows that the median element of the thyroid begins as a syncytial outgrowth from the



wall of the pharynx, having no intra-parenchymal framework of exoplasm and no lumen, while the lateral elements arising later in the development of the embryo have both an intra-parenchymal framework and a central lumen.

*Pig embryo 15-20 mm. in length*

The changes in the median element during this period are an increase in the parenchymal and exoplasmic syncytia and a rela-

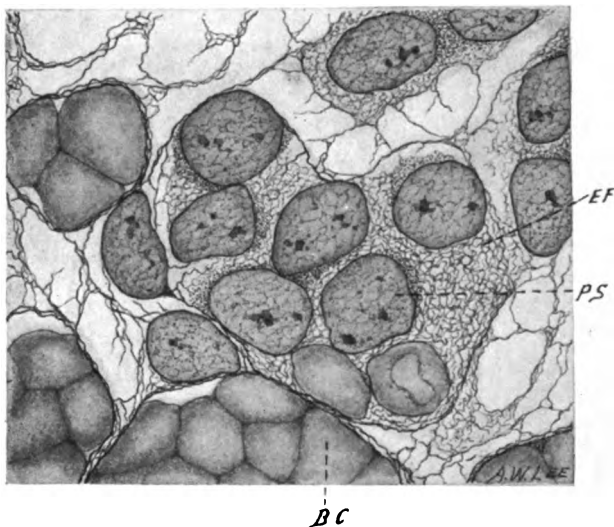


FIG. 6 Transection of thyroid of pig embryo 35 mm. long. Magnified 750 diameters. BC, blood corpuscles. EF, exoplasmic fibrils. PS, parenchymal syncytium.

tively greater increase in the number of blood vessels. In the lateral elements the rapid increase of nuclei has almost destroyed the intra-parenchymal framework, so that fibrils, cut ends of fibrils and nuclei of mesodermal origin, scattered here and there, are all that remain. The lumen has also been obliterated and these elements have gradually moved towards and finally united with the median element, so that in embryos 20 mm. in length the thyroid gland is a single mass. But on account of the latter origin

of the lateral elements they have not yet been invaded by blood vessels and can therefore be readily distinguished from the median element.

*Pig embryo 20-34 mm. in length*

Rapid growth accompanied by comparatively gradual changes mark this period of development. The restoration of the intra-parenchymal framework of exoplasm in the lateral parts and the completion of the framework in the median part take place. The increase of the vascular system in the latter is so rapid that in most embryos blood vessels appear to form the greater part of this portion of the gland. The invasion of the lateral elements by the vascular system begins in embryos 26 mm. long and proceeds slowly, so that in pigs 34 mm. long the greater vascularity of the median part still sharply differentiates it from the others.

*Pig embryo 35 mm. in length*

In sections stained by Mallory's method or with hematoxylin and congo red, the peri-glandular connective tissue has all the forms of nuclei usually found during the transformation of endoplasm into exoplasm and of exoplasm into fibrillae. The large vesicular variety of nuclei predominates but the small darker staining form is abundant. There is a definite capsule varying in density. Laterally, where it is crowded between the parenchyma of the gland and large blood vessels and dorsally, where it lies between the parenchyma and the trachea it is more dense than ventrally where the pressure is less.

Within the capsule the connective tissue syncytium permeates that of the parenchyma, forming an intra-parenchymal framework of exoplasmic fibrils and nuclei of the small dark-staining variety. Probably the large vesicular nuclei are also present but are not differentiated from the nuclei of the parenchyma. The interlacing fibrils of exoplasm that form the intra-parenchymal framework are continuous with those of the capsule and with those of the walls of the blood vessels within the gland (fig. 6).

These vessels are still much more numerous in the median than in the lateral elements. This is, however, the last stage of the series in which this differentiation is found.

Beginning with embryos of this size, the method of pancreatic digestion already described may be used with advantage in studying the development of the connective tissue framework. This method verifies the facts already established by the study of undigested, stained material.

The digestion of sections for a few hours removes all nuclei, both of the parenchymal and of the connective tissue syncytia, leaving undigested the stroma of the red blood corpuscles and the fibrillated exoplasm. The extra-parenchymal exoplasm shows a fine reticular structure which by condensation forms the capsule of the gland (fig. 7). The further development of this capsule is similar to the process described by Flint for that of the submaxillary gland.

Within the gland the fibrillated exoplasm forms a network with round or oval meshes approximating in size one or more of the parenchymal nuclei in undigested specimens of the same age.

*Pig embryo 45 mm. in length*

So far serial sections of the embryo have been used, but beginning with this stage the gland is removed before fixation. It is a small, approximately spherical mass, about .5 mm. in diameter. The development of the vascular system has been more rapid in the peripheral than in the central portion of the gland, obliterating the distinction that has hitherto existed between the parts formed from the lateral and median elements.

In the periglandular connective tissue many of the nuclei are of the large vesicular type, strongly resembling those of the parenchymal syncytium, within the gland the connective tissue nuclei are smaller and stain more deeply. The uniformity and continuity of the intra-parenchymal framework is beginning to disappear, while definite thickenings of this framework, here and there, foreshadow the formation of the follicular walls. There is no other indication of follicles; the cords of cells have no constrictions nor

any lumen. However, there are in the parenchymal syncytium occasional droplets of colloid between the nuclei. This colloid is not formed by the degeneration of nuclei, as described by Wölfler, for the parenchymal nuclei have a perfectly normal appearance.

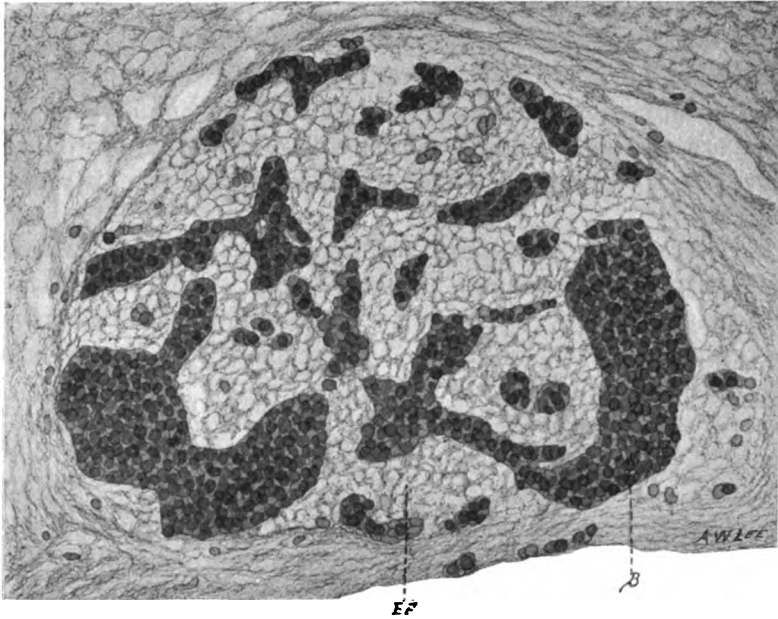


FIG. 7 Transection of thyroid of pig embryo 35 mm. long. Digested on the slide, stained with methylene blue. Magnified 187 diameters. B, undigested red blood corpuscles. EF, intra-parenchymal framework.

ance. In pigs then the appearance of colloid precedes the formation of the follicle, and is produced by the activity of the parenchyma (fig. 8).

*Pig embryo 60 mm. in length*

Sections stained with hematoxylin and congo red show that the parenchyma still exists as a syncytium, but occasional nuclei show more or less isolated masses of protoplasm about them. There are, however, as yet no cell membranes. Mallory's stain emphasizes the connective tissue and shows clusters of parenchymal

nuclei surrounded by stronger strands of fibrillated exoplasm. The rapid increase of parenchymal nuclei has still further broken down the mesodermal network, but strands of exoplasm may still be seen scattered here and there among the nuclei. Drops of colloid have increased in number and size, but there are still many masses of cells in which there is no appearance of colloid (fig. 9).

There is no evident determining factor as to where these drops of colloid appear. They may be separated by one or by many nuclei, or they may be close together with only a bit of protoplasm intervening; they may occur close to blood vessels or more remote from them.

Digested specimens confirm the story already told. Isolated areas with stronger strands of connective tissue fibrils around them contain a reticulum of finer fibrils. In some of these areas where the continuity has been broken, the finer fibrils have been washed away during preparation.

*Pig embryo 70 mm. in length*

At this stage are found the first follicles with completed walls (fig. 10). These are few in number and only seen in sections stained by Mallory's method. Digested specimens show a framework enclosing irregular spaces of varying sizes and shapes, none of which are as small as the follicles. Delicate strands of fibrillated exoplasm extend from this framework into the spaces forming incomplete partitions, which ultimately become follicular walls. These first formed follicles differ from those in the adult in the syncytial character of the epithelial lining, which is a single layer of nuclei surrounded by protoplasm. Between some of these nuclei fibrillated exoplasm may still be seen (fig. 10).

The colloid drops are increasing in number and size throughout the gland and the rapid increase in nuclei is completing the breaking down of the intra-parenchymal network.

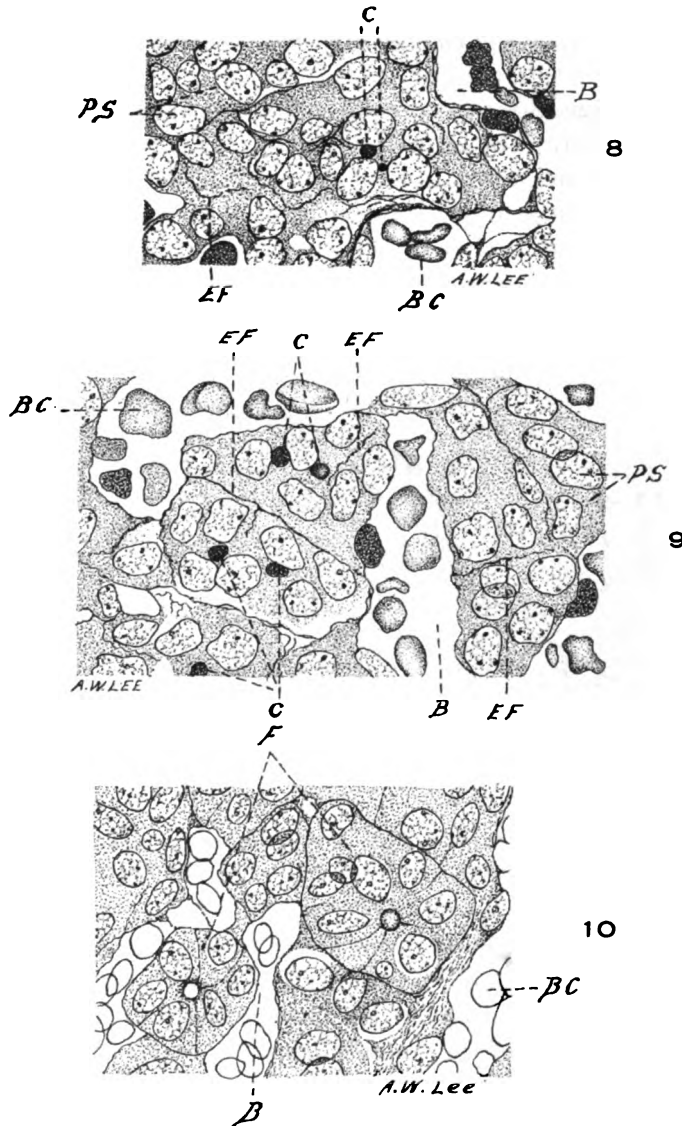


FIG. 8 Section of thyroid of pig embryo 45 mm. in length. Magnified 500 diameters. B, blood capillary. BC, blood corpuscles. C, drops of colloid. PS, parenchymal syncytium. EF, exoplasmic fibrils.

FIG. 9 Section of thyroid of pig embryo 60 mm. in length. Magnified 500 diameters. B, blood capillary. BC, blood corpuscle. C, drops of colloid. EF, exoplasmic fibrils. PS, parenchymal syncytium.

FIG. 10 Section of thyroid of pig embryo 70 mm. in length. Magnified 500 diameters. B, blood vessel. BC, blood corpuscle. F, follicle.

*Pig embryo 100 mm. in length*

At this age the secretion of colloid is abundant throughout the syncytium. The growth of connective tissue has been rapid, resulting in the formation of many complete and incomplete follicles. In some follicles the nuclei are not arranged in a definite outer layer so that they do not encircle the colloid, which is separated in these places from the wall of the follicle by protoplasm alone.

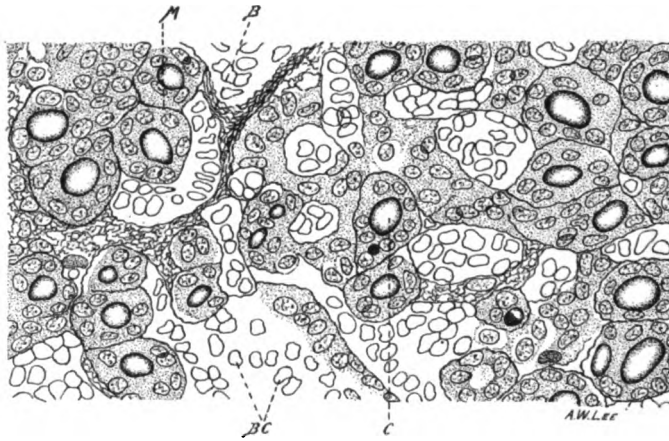


FIG. 11 Section of thyroid of pig embryo 100 mm. in length. Magnified 372 diameters. B, blood vessel. BC, blood corpuscle. M, follicle wall. C, colloid.

The size of the colloid drops seems to bear no definite relation to the development of the connective tissue wall of the follicle, many of the larger drops lie in masses of nuclei without follicular walls, while some of the smaller drops are enclosed in a complete follicle.

Fibrillated exoplasm is now rarely seen between the nuclei assembled around a drop of colloid. It is more common among the masses and columns of cells not differentiated into follicles, but even here it is disappearing.

Some blood vessels have developed walls of considerable thickness from which large strands of connective tissue pass into the parenchyma in such a way as to suggest future lobulation.

No differentiation is now to be seen between the central and the lateral parts of the gland in vascularity, colloid formation or connective tissue development (fig. 11).

*Pig embryo 100-140 mm. in length*

During the period in which the embryo is increasing in length from 100 to 140 mm. the rapid formation of follicles by the growth of septa, and the increase of colloid continue, accompanied by a corresponding increase in the syncytium of the gland. In embryos about 140 mm. in length distinct cell outlines are first found in the parenchyma. These appear in the older follicles and are not seen in the undifferentiated cell-masses which are, however, not numerous. Hence it is clear that colloid is formed for a considerable time while the gland is a syncytium.

*Pig embryo 170 mm. in length*

The division of the syncytium into follicles is essentially complete. Branching follicles, such as Streiff as described in man, now begin to appear and are found in all later stages. The transformation of the parenchymal syncytium into cells has proceeded rapidly. Digested sections show the follicle walls to be formed of reticulated connective tissue, the fibrils of which may readily be seen with higher powers.

In pieces of the thyroid prepared according to Flint's method, stained with aniline blue, mounted in glycerine, the framework of the gland may be seen to a considerable depth. These preparations show septa of connective tissue passing from the walls of some blood vessels to become continuous with the walls of other vessels or with the capsule of the gland.



*Two-day pig*

The transformation of the syncytium into cells is completed, and in section the gland is seen to be made of follicles, the definite inter-follicular framework carrying a rich supply of blood vessels, and masses of cells that have been called resting cells lying here and there between the follicles. The parenchymal

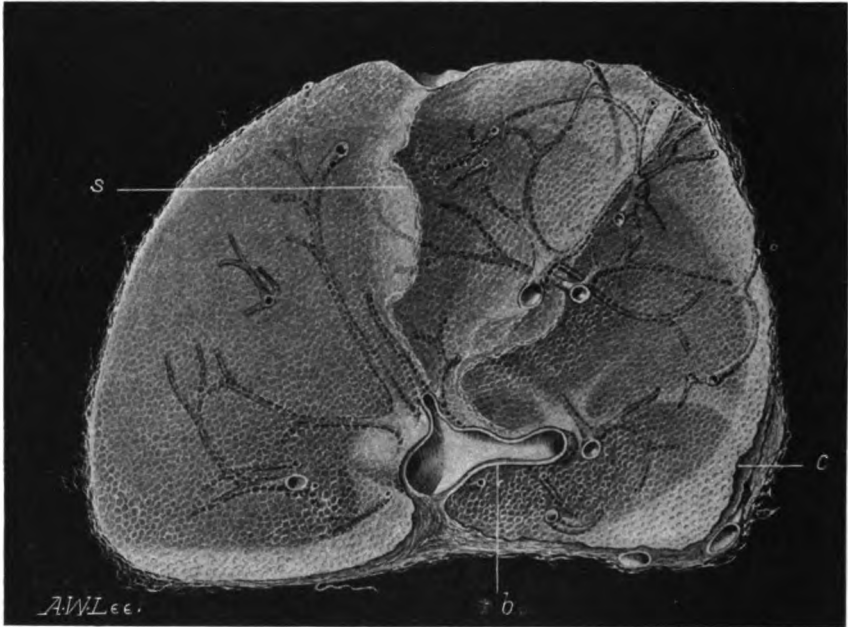


FIG. 12 Transection, 1 mm. thick, of thyroid of pig 2 days old. Magnified 31 diameters. Mounted in glycerine, and drawn with the aid of the stereoscopic microscope. B, blood vessel. C, capsule. S, septa.

epithelium is of the low cuboid variety with no differentiation into chief and colloid cells as is described by Langendorff.

The follicles are losing their earlier globular shape and are becoming more polyhedral in form. Digested sections show a marked increase in the number of connective tissue fibrils in the follicle wall, which results in a much finer meshed reticulum. Block digestion of transections of the entire gland shows an almost kid-

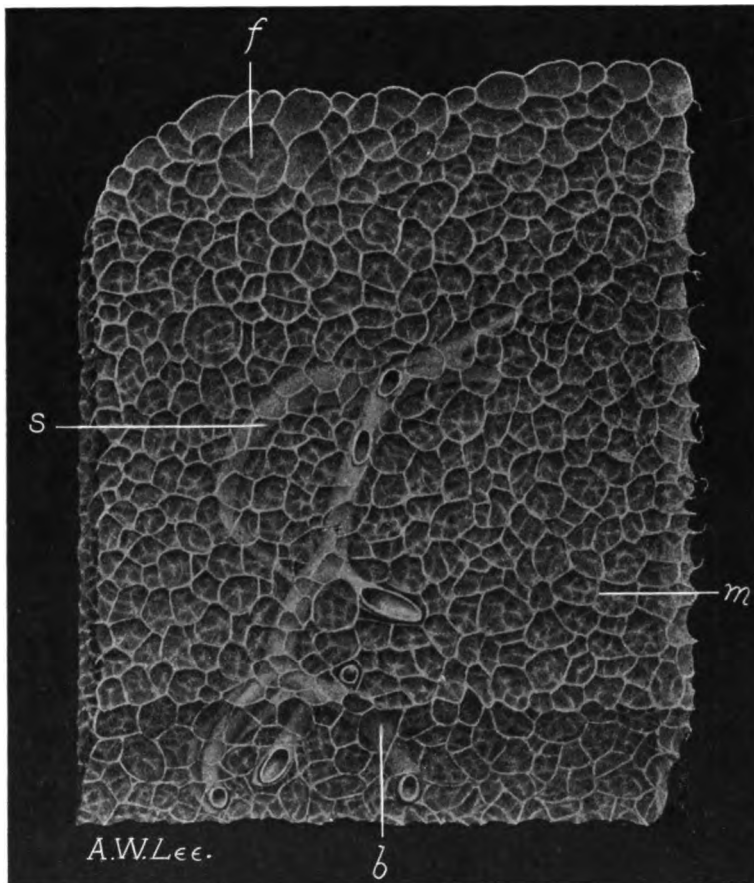


FIG. 13 A digested free hand section about 1 mm. thick of thyroid of adult pig. Drawn with the stereoscopic microscope and reflected light. Magnified 25 diameters. B, blood vessel. F, follicle. M, follicle wall. S. connective tissue septum.

ney shaped outline, the connective tissue entering at the hilum with blood vessels, and apparently dividing the gland into irregular lobules. These septa, however, are not continuous throughout the gland so that the lobulation is incomplete. The size and shape of the follicles is well shown (fig. 12).

*Adult pig*

The follicles have increased in size and number and the consequent crowding has further developed their polygonal form. Their walls have increased in thickness and their component fibers are larger and stronger. These changes are readily seen with the stereoscopic microscope in sections 1 mm. thick (fig. 13).

With greater magnification may be seen the connective tissue fibrils and the reticular structure of the walls as well as the coarser

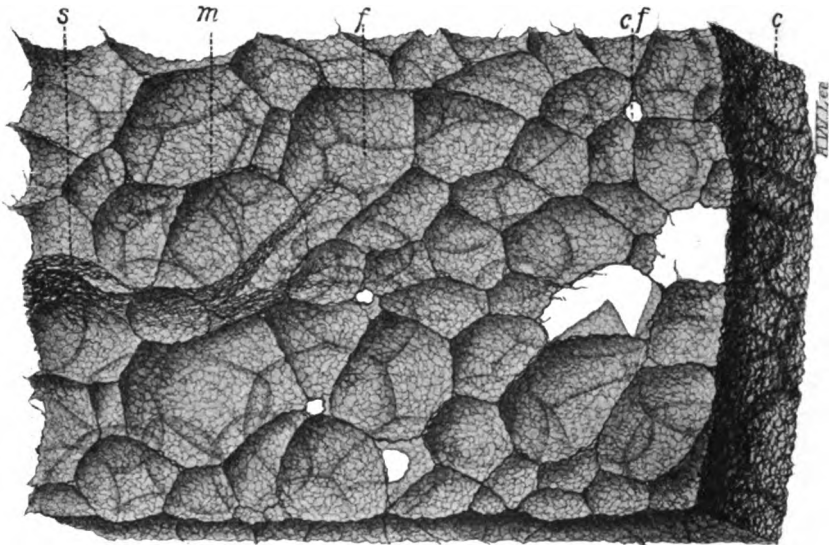


FIG. 14 Part of 13 highly magnified. C, capsule, cf, cut follicle. F, follicle. M, follicle wall. S, septum.

network of the septa and of the capsule. There are also in some preparations small round and oval openings in the follicular walls distinctly unlike the openings between the meshes of the connective tissue (fig. 14).

## CONCLUSIONS

The median element of the thyroid of the embryo pig in the earlier stages, is a distinctly bi-lobed syncytium with neither an inter-nuclear mesodermal framework nor a lumen. The meso-

dermal syncytium enters the parenchymal syncytium in two ways: it is carried in by blood vessels, and passes directly in from the surrounding mesoderm. The vascularization of this element takes place in embryos about 14 mm. in length.

The lateral elements are also syncytial in character, but have an intra-syncytial framework of exoplasm and a central lumen. This framework disappears after the union of the lateral and median elements, which takes place in embryos about 20 mm. in length.

The lateral and median elements can be distinguished by the difference in vascularization until the embryos are about 35 mm. in length.

The intra-parenchymal framework of exoplasm is present throughout the gland in embryos about 35 mm. long, but as such soon disappears.

Colloid is first formed early in embryonic life, before the formation of follicles and while the parenchyma is still a syncytium. In pig embryos colloid is not formed by cell degeneration.

The follicles, first found in embryos 70 mm. in length, are formed from the parenchyma by the ingrowth of connective tissue from the walls of blood vessels and from the capsule and by the strengthening of portions of the intra-parenchymal exoplasmic framework.

Epithelial cells formed from the parenchymal syncytium are seen first in embryos about 140 mm. in length. The transformation of this syncytium into epithelium is completed before birth.

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# NOTE ON THE PRESENCE OF THE FIFTH AORTIC ARCH IN A 6 MM. PIG EMBRYO

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## THREE FIGURES

In a paper printed in 1906, F. T. Lewis, after reviewing the observations recorded by previous authors and those made by himself, came to the conclusion that "The interpretation of the postbranchial body in mammals as a fifth pouch, and of the irregular vessels in front of it as a fifth arch are morphological speculations of much interest, in favor of which the preceding observations afford no certain evidence." The same author had also made the statement in a previous article that "The irregular small arteries around the fourth entodermal pouch do not, as Zimmermann believed, form a distinct aortic arch." To this view W. A. Locy has taken exception.

In 1907, Locy summarized the observations upon the vascular elements between the fourth and sixth arches as follows: "(a) A complete arch as an offshoot of the fourth and returning to it, (human, Zimmermann); (b) a complete arch connecting the fourth and pulmonic arches, (pig, Lehmann, Locy; rat, Tandler); (c) a complete arch from the truncus arteriosus to the pulmonic arch, (human, two cases, Tandler); (e) irregular vascular elements from the fourth arch, the aortic root and the pulmonary arch, (pig, Lehmann, Lewis; rabbit, Lewis, Lehmann; rabbit, in stages of degeneration of the arch, Zimmermann); and (f) a single vessel from the distal end of the pulmonic arch with unknown ventral connection, (sheep, Tandler)." Locy then adds, in opposition to the position of Lewis: "In the light of these observations it would

appear that the vascular elements between the fourth and pulmonary arch exhibit great variability, but there are recorded a sufficient number of cases of their aggregation into a complete vessel to justify the assumption that there is a fifth aortic arch in mammals, as in other vertebrates."

In looking over the Princeton series no. 126 of a 6 mm. pig, it seemed to me that there was present a fifth aortic arch on both sides of the embryo. In order to confirm this opinion, at the suggestion of Prof. C. F. W. McClure, a wax reconstruction was made, and such was clearly seen to be the case on the right side at least. As shown in fig. 1,<sup>1</sup> the first arch has already undergone considerable degeneration, while the second has almost entirely disappeared. The third, fourth and pulmonary arches are quite normal; the fourth is the largest, and the pulmonary has begun to give off the pulmonary artery. On the right side of the embryo there is a fifth arch which arises independently and connects with the fourth arch shortly before the latter enters the dorsal aorta.

This arch arises almost midway between the fourth and the sixth arches, there being a broad connection between its base and the fourth arch. It runs obliquely for a short distance from the aortic root toward the sixth arch and then turns at right angles to enter the fourth arch; but just before doing so it is connected dorsally with the sixth arch. From the turn on it narrows gradually up to a point just before its entrance into the fourth arch where it broadens out once more; this fact together with its beaded appearance would indicate the beginning of degeneration.

In the light of the work of Evans upon the development of the aortae a very interesting interpretation can be placed upon the structure of the fifth arch as shown here. Considering the connections between the fifth arch and the fourth and the sixth arches as enclosing foramina and taking into account a third foramen that lies in the arch itself, we have at the dorsal end of the arch the remains of a capillary plexus whose vessels are of a relatively large caliber; all traces of it at the ventral end have been lost. It

<sup>1</sup> My thanks are due to Mr. O. F. Kampmeier who kindly made the drawing from the reconstruction, and to Mr. C. F. Sylvester for helping me with the microphotographs.

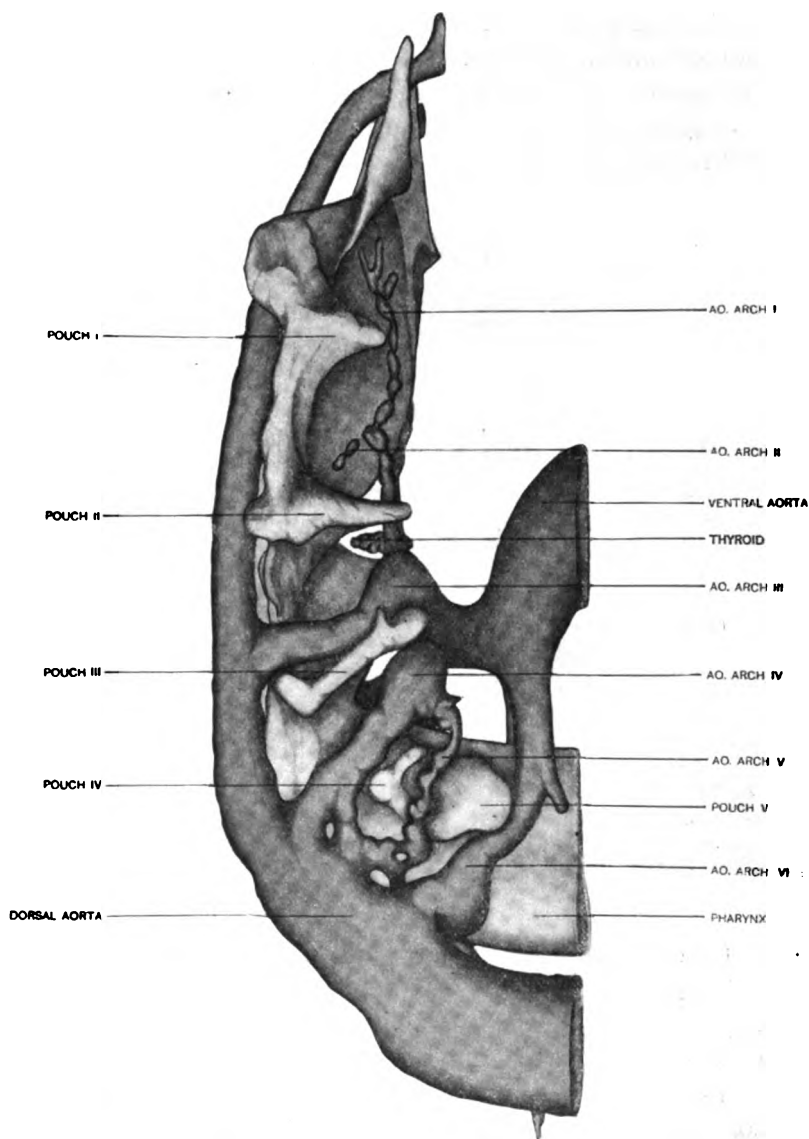


FIG. 1 Reconstruction of the pharynx and aortic arches of a 6 mm. pig embryo. Princeton Embryological Collection No. 126. Right side.



is from this plexus that the arch arose. At its dorsal end the formation of a single vessel from the plexus is not completed, although the main channel is clearly marked out. At the ventral end, on the other hand, not only has the single main vessel been established, but degeneration has already set in, this being due to the fact that most of the blood passes through the fourth arch. This

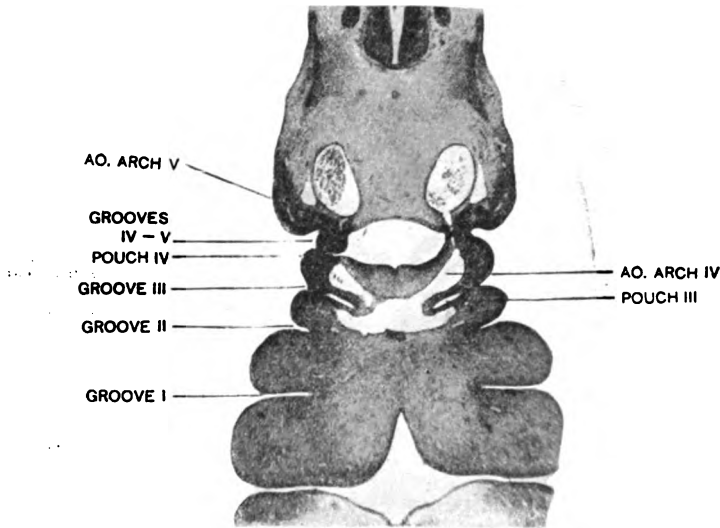


FIG. 2 A 6 mm. pig, Princeton Embryological collection, series 126, section no. 98. This section, through the region of the pharynx, shows the fourth pharyngeal pouch and the five ectodermal grooves on the right side. The fifth arch is seen in cross section just below the right dorsal aorta.

disuse of the fifth arch would naturally cause the degeneration of the ventral half of the vessel very soon after its formation, in fact probably before the dorsal half has been fully crystallized out. Moreover, there is no reason to believe that this process of crystallization would continue at the dorsal end after degeneration had once commenced below.

To my mind this affords a very clear explanation of why so many instances have been observed of irregular vascular elements lying between the fourth and the pulmonary arch and for the most

part connected with the aortic root. The embryos were probably killed after the ventral portion of the arch had degenerated, or perhaps, as was most probably the case, the plexus had disappeared at that point without having formed a single main channel. But it is to be noted that very probably the broad portion of the fifth arch at the point where it joins the fourth arch is to a

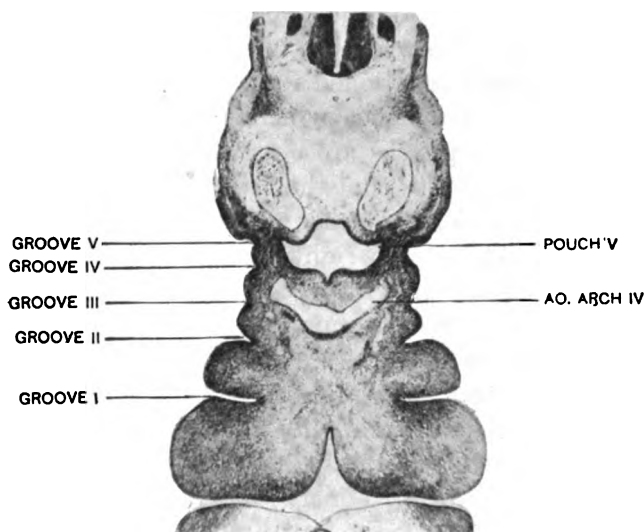


FIG. 3 A 6 mm. pig, Princeton Embryological collection series No. 126, section 102. This section shows the five ectodermal grooves more clearly than they are shown in fig. 2. The fifth pouch on the right side is seen to correspond to the fifth groove. The fifth arch is also shown here in cross section but further away from the right dorsal aorta than shown in fig. 2.

certain extent comparable to the spurs described by Coulter in the cat embryo. Its persistence would seem to indicate that whether or no a main channel is formed from the plexus there is a stimulus for the blood to flow in that direction.<sup>2</sup> On the left side of this embryo the appearance of the vascular elements resembles in general some of the cases described by Miss Lehmann, there

<sup>2</sup> In this particular case there can be no doubt that the channel formed was used as an actual blood passage, as blood corpuscles were found to be present in it.

being a narrower and shorter vessel running from the aortic root to the fourth arch, together with other small and irregular vascular remnants. Here, where in all probability a main channel was never formed, there is a spur on the fourth arch similar and corresponding to the broadened ventral end of the fifth arch on the right side.

In further support of the statement that we are dealing here with a complete fifth arch is the fact that there are five pharyngeal pouches present, between the fourth and fifth of which lies the arch. In the paper mentioned above, Miss Lehmann also described five pouches, but Lewis is inclined to doubt that the postbranchial body (fifth pouch) arose as a true pharyngeal pouch. It is true, as he points out, that the fifth pouch never comes into contact with the ectoderm, but here there is very strong evidence that it is a true pouch. On the right side of the embryo from which the reconstruction was drawn there are five distinct ectodermal grooves present, one corresponding to each pharyngeal pouch. It is to be noted that the five grooves are found on the same side as the complete fifth arch, while on the left side, where there is a smaller and less complete arch, only four ectodermal grooves are to be seen. This is shown in figs. 2 and 3, which are photographs of two transverse sections, 15 micra thick, through the region of the pharynx. Fig. 2 shows the fourth pouch on the right side in contact with the ectoderm as well as the five ectodermal grooves. Fig. 3 gives a clearer view of the five grooves and also shows the fifth pouch almost in its entirety. Other 6 mm. pig series of the Princeton collection were examined, but none of them showed either five ectodermal grooves or a complete fifth arch on either side. This one clear case, however, of the presence of a fifth ectodermal groove, together with the well known fact that the fourth pouch and the postbranchial body arise from the pharynx independently of each other, indicates that the latter was originally a true pharyngeal pouch, *i.e.*, the fifth.

It is clear that here we have a series of sections of a 6 mm. pig which is particularly advantageous. On the right side of the embryo not only is there a more complete fifth arch than has hitherto been described for the pig, but there are also five pharyngeal

pouches and five ectodermal grooves corresponding to them. The left side, on the other hand, shows an arrangement of the vascular elements and the pharyngeal pouches which resembles in general those described by previous writers. In view of this evidence the existence of a complete fifth arch in the pig can hardly be called into question any longer and, in the opinion of the writer, those vascular elements so frequently found between the fourth and the sixth arches must be interpreted as the remnants either of a fully developed or a potential fifth arch.

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## THE WISTAR INSTITUTE, DEPARTMENT OF EMBRYOLOGY

At a conference of ten anatomists held at The Wistar Institute, on April 11, 1905, it was recommended that the principal object of the Institute be research, and that the work shall be in the fields of neurology and embryology. This action was approved by the Board of Managers a week later and was incorporated in a letter by General Wistar to these scientists, inviting them to become members of a permanent Advisory Board of Anatomists of The Wistar Institute (see Bulletin No. 1 of The Wistar Institute of Anatomy and Biology, September, 1905).

The Advisory Board considered it best to proceed with the organization of the department of neurology first, and accordingly Professor Donaldson of Chicago was elected head of this department. With him were associated Dr. Streeter and Dr. Senior, both of whom were subsequently called to chairs of anatomy in important universities. The work of the department of neurology is now well established, as outlined in Bulletin No. 1, and by Dr. Greenman in the *Anatomical Record* of June, 1907.

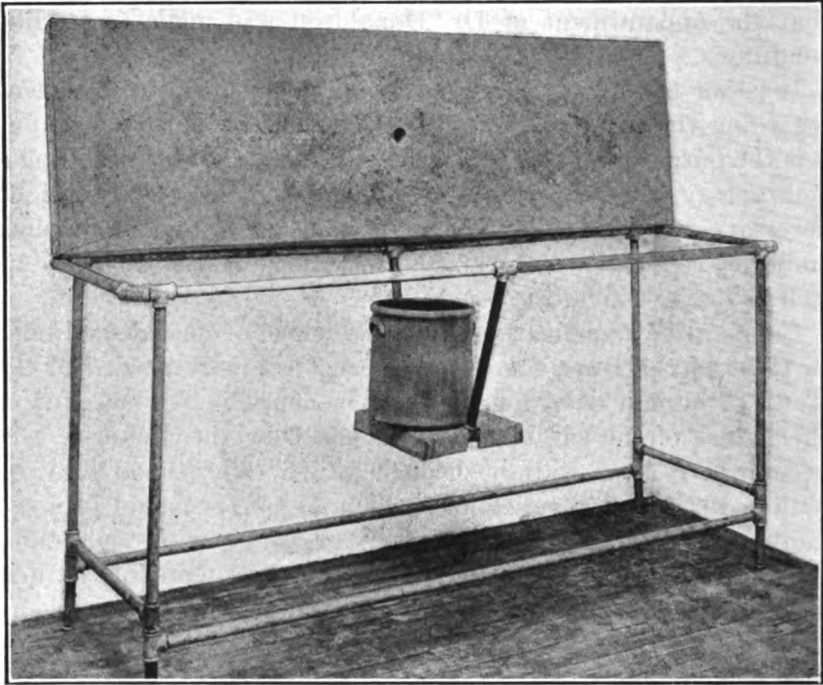
In the meantime, funds have accumulated which enable the Institute to support properly a department of embryology, and at the meeting of the Advisory Board held on April 25, 1910, Professor Huber of the University of Michigan was nominated for this office. Prof. Huber has been secretary of the medical faculty of the University of Michigan since 1897 and director of the laboratory of histology and embryology since 1898. In 1901 he was elected secretary of the American Association of Anatomists, and he has been an editor of the *American Journal of Anatomy* and of the *Anatomical Record* since their foundation.

Dr. Huber will reside at the Institute from March to September in 1911 and 1912, after which it is hoped that he may devote his entire energies to the work of the Institute. It may be recalled

that the appointment of Dr. Donaldson was made under like conditions.

It is not possible at this time to make any definite statement regarding the work of the new department of embryology, but it is the purpose to make it largely a place for the study of human embryology and its relation to anatomy in general. It will be necessary to make collections of human and other mammalian embryos, especially early stages, for study at the Institute as well as for investigators elsewhere.

Coöperative investigations and conferences of specialists similar to those of the Brain Commission are even more needed in the study of human development than in neurology on account of the nature of the subject. It is hoped that the Institute will become a central station through which information may be obtained concerning collections of human embryos and to assist individual investigators to secure, by exchange or through loans, the necessary specimens. However, the chief work of the new department will be at the Institute, but it will assist others and coöperate with them in every possible way.



## A SIMPLE DISSECTING TABLE

H. R. WAHL

The dissecting table illustrated above was designed for use in the Anatomical Laboratory at the University of Wisconsin. It consists of a frame-work made of ordinary one-inch iron piping and of a movable galvanized iron top. The legs of the table are braced by iron piping placed six to eight inches from the floor. These braces not only strengthen the table but also provide a convenient foot-rest for the student while dissecting. The great advantage of arranging the piping as illustrated lies in the fact that no obstacles are offered to the knees of the student while sitting on the stool during dissection. The galvanized iron top is six and one-half feet long, two feet wide, and when placed on the frame is three feet above the floor. It is grooved in such a way that the sides slope gently towards an opening in the center. Below the opening in the center a rack is suspended for holding the drainage jar. The table is inexpensive and can readily be built by any plumber or steam-fitter.

## A CRITICISM OF OUR MODERN TEXT-BOOK OF ANATOMY

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It may be claimed that the criticism the writer is about to make applies more to the previous training of the medical student in high schools and college than it does to the text-book of anatomy, but even admitting that it does, there is no reason why the authors of text-books should not consider the conditions as they are. It seems to make but little difference whether the student is a college graduate or whether he has had but two years of college training, the fact remains that he does not know the meaning of most of the names in the anatomical terminology. It is no exaggeration to say that even such simple words as *tuber*, *sartorius*, *buccinator* and even *caput*, do not convey anything to the student. Latin and Greek are taught as methods in the high school and college and little stress is laid upon the importance of the vocabulary that the student of these subjects should naturally acquire. It is evident that he forgets the vocabulary in order to leave his mind more receptive for such subjects as mathematics, chemistry and biology. It is deplorable that most students who intend to study medicine do not learn enough Greek to be able to at least read the Greek characters. However, it is not within the scope of these remarks to suggest reforms in the early training of our students; it is the writer's intention to merely call attention to the status quo, and point out where our present text-books fail to attempt to remedy the evil caused by the early training of the medical student.

Four hundred years ago, Jacobus Sylvius, following the example set by Galen, gave names to most of the muscles of the human body. In doing so he considered their origin (*brachialis*), their structure, (*biceps*, *membranosus*), their shape (*serratus*), and their size (*magnus*, *brevis*). Previous to his time numerals were applied. It goes without saying that it made the memorizing of anatomical terminology considerably easier. That Sylvius should have used Latin and Greek words is evident. Latin was the language of the learned, and all scientific treatises were written in Latin. From Hippocrates he took the Greek words. In those days every student understood the meaning of the terms and it is unlikely that any should have made the mistake of calling the *serratus anterior*



muscle the latissimus dorsi, as some of our students do to-day. Yet most students would resent the inference that they do not know the meaning of the term latissimus.

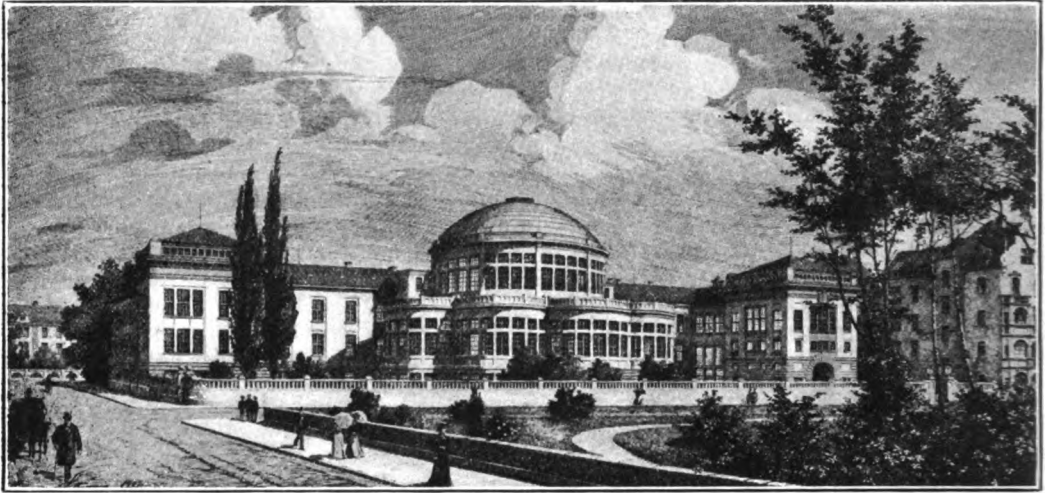
What is the condition of affairs to-day? It has been the writer's habit frequently to ask the student the meaning of the terms thyreoid, ilium, azygos, etc., and of prefixes, mesa, meta, supra, semi, etc. The ignorance displayed by the student was sometimes amazing and one wonders how it was possible for him to graduate from high school, let alone college. Ask a student of average ability to demonstrate the extensors of the wrist and fingers. He will probably do so in the following order: extensor carpi radialis longus, ext. carp. rad. brevis, extensor digitorum communis, ext. digiti quinti proprius, ext. capri ulnaris. Make him begin his demonstration on the ulnar side and see the result. It has the same effect as asking a person to say the alphabet backwards. This means nothing else but that the student has memorized the muscles in a certain order, and he would be far better pleased if, instead of having long Latin names attached, the muscles were known as muscles I, II, III, etc. Now in the cases cited there is no name which ought to leave any doubt in the student's mind as regards its meaning, and a demonstration ought to be very simple provided the muscles have been dissected out from their origins to their insertions. The student, however, has so accustomed himself to take most of the names found in the anatomical terminology as so many meaningless words, that he even ignores the meaning of those which have been anglicized and are used by the layman. Thus the term fascia is defined by the student, and unfortunately by a number of anatomists, as a layer of areolar connective tissue. The derivation from the Latin fascia meaning band or bandage is entirely lost sight of, although it gives us the true meaning of the term. Pertioneum is another instance. Let the student know the derivation, which means to stretch around, and he will not get absurd notions of its being thrown into folds without the intervention of some organ or other structure. Let the student adhere to the idea of "stretching" and many wrong impressions will be dispelled. The derivation of the term oesophagus explains its function, of parotid its position, of thyreoid its shape, etc.

The result of this ignorance about the derivation of words is far-reaching. In the clinics the same student may characterize a condition whose name ends in "itis" as a swelling, and the meaning of such important prefixes as haemo, rhino, ileo are absolutely lost to him. He must learn the name of every disease just as he would the name of so many persons. It never occurs to him that about 90 per cent of all the names are descriptive of the disease. Even if most of the blame of this condition is to be laid on the high school and college, there is no valid reason why we anatomists should not begin with a training that is of such vital importance. We have other duties in the dissecting room besides the teaching of anatomy, such as training the student's power of observation, his self-control, his tactile sense, and trying to inculcate a critical or scientific attitude. In a word, we strive to make the student fit to enter upon the study of medicine. It seems but a small additional

duty to make the student realize the importance of word-derivation. To make this the subject of a lecture-course is not feasible with the present crowded schedule in our medical schools. It is sufficient for the instructor to repeatedly call attention to derivations, provided the student can find a reliable source of information. What would be simpler than to have this information incorporated into our text-books. It was done in some of our older text-books. The omission is probably due to the publisher who is instilled with fear at the very sight of a Greek letter.

The conditions in Germany are far different. It is attributable partly to the better elementary training in the classic languages and partly to the custom prevailing among the authors on anatomical subjects to use German instead of Latin names. There is no reason why we should not follow the example of our German confrères in at least the latter respect. Why not speak of the tailor muscle, the straight abdominal muscle, the two-headed muscle, the calf-muscle, the medial skin nerve of the fore-arm? It is evident that it cannot be carried through as consistently as is possible in German, because we have but comparatively few Anglo Saxon roots to build upon. Yet the interspersing of a few of such terms will take away a great deal of the oppressing formality of the present text-book and make it far more pleasant reading matter for the students. If any one should doubt that Anatomy can be made the subject of most delightful literature he ought to read the text-book of Hyrtl.

The diadactic importance of these suggestions cannot be over estimated. The student will lose that antagonistic attitude toward the subject of anatomy because this attitude is only bred in ignorance. To the average American student of Anatomy at first, especially if he begins with a lecture course in osteology, means merely the memorizing of numerous names. The instructor speaks an unfamiliar language,. With the aid of our next text book let the student's attention be called to common prefixes and suffizes, and the derivation of terms. His horizon will be widened immediately, he will use the terms with greater assurance and familiarity and incidentally learn something of the history of anatomy.



**FIG. 1** The anatomical institute in Munich. Histology is taught in the dome over the dissecting room.

## BOOK REVIEW

**DIE NEUE ANATOMISCHE ANSTALT IN MÜNCHEN.** Von Dr. J. Rückert. 109 Seiten und 18 Tafeln. Wiesbaden: J. F. Bergmann. 1910.

Professor Rückert's account of the new anatomical laboratory in Munich is of interest to Americans because it will be of great value to those who have similar buildings to plan. We are now in the midst of a development of medical schools which calls for the construction of first-class laboratories by our leading universities. Those who have this task before them will welcome good literature upon laboratory construction.

The first anatomical laboratory built at Munich was planned by Döllinger in 1824. (It may be stated that the anatomical laboratory at Johns Hopkins University is in many respects a reproduction of Döllinger's laboratory.) Soon this laboratory proved to be inadequate, and in 1855 it was rebuilt by Bischoff who made it about as large as

the present beautiful laboratory of the Harvard Medical School. Now there is a third stage in the development of medical education in Munich which has called for this "palace," as the proper home for anatomy in

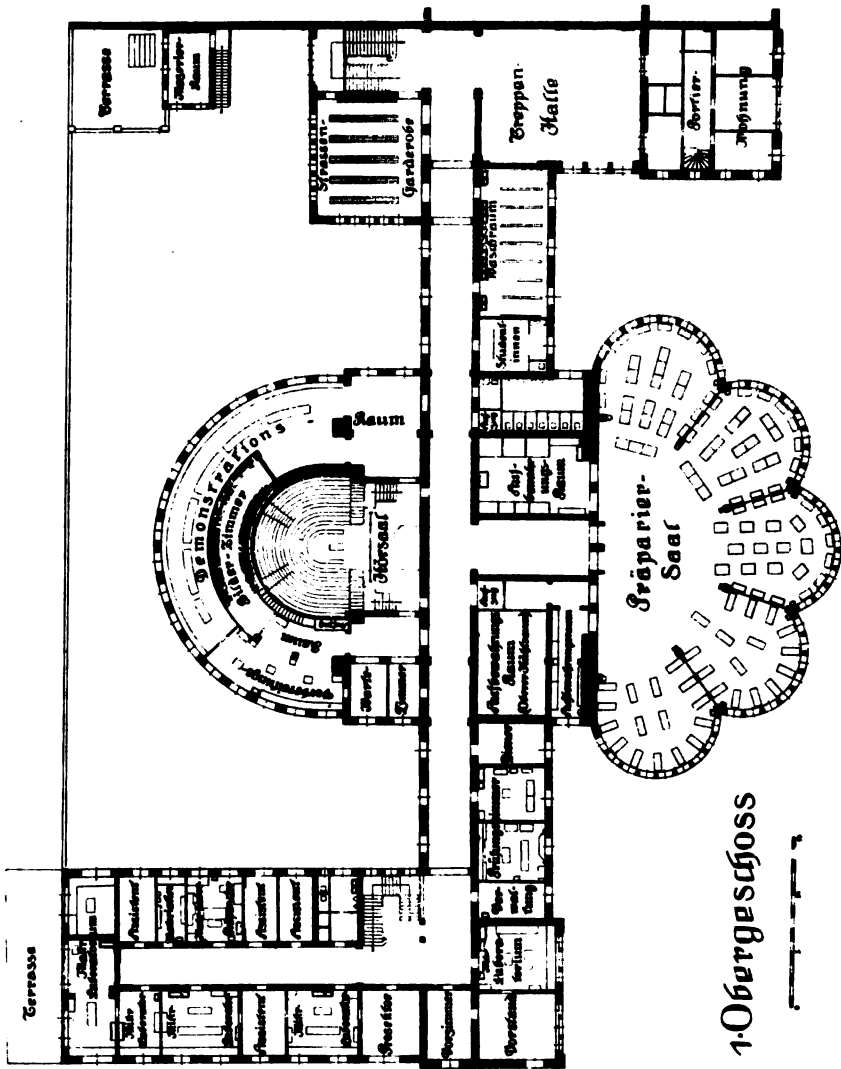


Fig. 2. Plan of the first main floor showing the distribution of space devoted to the teaching of gross anatomy. The scale is in meters.

a large and great university. This new anatomical institute is an irregular-shaped building, four stories high, and covers an area of about 200 by 300 feet. Like the Harvard laboratory, which is by all odds

our best example, it is a magnificent structure perfect in every respect for the work which is to be done within its walls. However, there are marked differences in the methods and teaching in these two laboratories, which makes Rückert's report especially valuable for the solution of problems of anatomical teaching soon to be encountered by our larger medical schools.

During the past academic year about 2000 students received instruction in anatomy in the Munich laboratory. The main courses given were as follows: Practical anatomy, 900 students; lectures on gross anatomy, 600; lectures on art anatomy, 600; lectures on histology and embryology, 500; exercises in histology, 300; and histological technique, 200. There were also numerous smaller and informal courses given. Since they believe in Munich that the larger a medical school is the better it should be, they have constructed a building in which both large-class instructions and individual instruction are given. One is impressed with what perfection they have met the difficulty of managing large groups, the equipment, machinery and service at the professors' disposal being the best in every respect. The building is immaculate, hygienic and aesthetic, with an ample budget for maintenance and for scientific work.<sup>1</sup> Those who have visited the laboratory are greatly impressed and leave with a feeling that Professors Rückert and Mollier have solved their problem in a most satisfactory way.

The report is detailed and is not only a good morphological but also a physiological description of the building. It is also educational. For these reasons it will be of use to all teachers of anatomy. All sub-departments of anatomy are well represented. The equipment for the preparation of all kinds of anatomical specimens, sections of embryos, museum specimens, skeletons, drawings, photographs, X-rays, experi-

<sup>1</sup> The following figures were obtained from Professor Mollier:

The building cost nearly.....	\$500,000
During the year 1909-10 the budget was, not including salaries.....	27,000
<hr/>	
General service, business manager, porter, technician, mechanic, stoker, etc.....	\$2,200
Heat, light, etc.....	7,600
Library.....	300
Assistants—	
Gross Anatomy (8).....	4,700
Histology (5).....	2,300
For materials, instruments, chemicals, artists, modelers, printing, photography, laundry, scientific apparatus, etc.	
Gross Anatomy.....	7,200
Histology.....	2,700
<hr/>	
Total.....	\$27,000

mental anatomy, etc., is at hand. There is an excellent collection for teaching and a beautiful display of specimens and models for the general public. As is well known, art anatomy is well cultivated and many students of art receive instructions here. In general one wing is devoted to the beautiful entrance and the reception of the students, the other wing to the staff and research, and the center of the building to the teaching of large classes (see figs. 1 and 2). In the sub-basement there are all sorts of cellars, coal bins, boilers, apparatus for ventilation. Above, in the basement there is the public museum, cold-storage rooms for cadavers and for the teaching collection, machine shops, and six residences for servants. The first main floor includes in the center the main lecture room and the large dissecting room built in the shape of a clover leaf. In one wing there are cloak rooms, dressing rooms for both men and women, etc., and in the other, rooms for the staff in gross anatomy. The dissecting room and the lecture hall extend upward through the second main floor, which contains also many rooms for the staff in histology and embryology. The large teaching room for histology is in the dome of the building over the dissecting room on the third main floor. Here there are also a very large demonstration room for histology, dark rooms, photographic rooms, X-ray rooms, etc. Everything is centralized as the main method of teaching is by means of lectures and demonstrations.

The bulk of students are passive; they listen to lectures and they witness demonstrations. Only the better students and members of the staff are active, study nature and are self-instructed, as Döllinger and von Baer would have it, and as is only possible for all students in a small laboratory. Both active and passive methods of teaching anatomy are used upon the same students only in exceptional cases. All in all, they have met the difficult problem of teaching large groups of students exceptionally well in Munich and one cannot study this report nor visit the institute without being greatly impressed with their methods.

Again using Harvard as our best example of an anatomical department with which to compare that in Munich, we find in each two eminent men as professors, and the entire teaching staff numbers about the same in each. However, we find at Munich from ten to twenty times as many students to be taught anatomy as at Harvard. The total cost of teaching this subject at Munich is by no means ten times as great as at Harvard but the equipment for teaching is much greater and the professors receive more than twice the salary that is paid at Harvard. It is clear that in Munich the teaching is fully centralized in the hands of the professors who teach very large classes, while at Harvard it is in sections, and is largely individual and inductive. In Munich all of the elementary instruction is given by the professors, which is not the case in America generally. The Munich method of instruction is more economical than ours, for there the salary of professors is large enough to induce eminent men to do practically all of the work. Therefore, the laboratory is built to aid the professor in his teaching, much more than other members of the staff.

Students who cannot help themselves in Munich are not stuffed through a Nuremberg funnel; recitations and quizzes are unknown. The teaching is adjusted to the average student, but it is scientific and those who cannot comprehend it are not whipped into line. Just at this point they save enormously in energy and do not commit the sin of trying to make physicians out of the unworthy. Students who desire more instruction than is given in the regular courses, that is, those of ability and originality, are given every encouragement (p. 48). Those who cannot comprehend are constantly eliminating themselves, for they drop out, while the great men of medicine are developed from the group of talented students, through encouragement. It follows that students are not forever being pulled up by the roots to see whether they are growing, but a process of natural selection is at work. Mutations, when recognized, are, however, always preserved.

What has been said above concerns the professors and the students whose work is located mainly in the center of the building at Munich. But one wing, which is a large laboratory in itself, is reserved for the staff and for research. Here we find the very soul of a laboratory, for it is here that the assistants and advanced students do their work. The greater part of the teaching is done at Munich by the professors, which in a way protects the assistants whose responsibility is mainly to themselves and to science. They are not worked to death with routine teaching but are permitted to become scientific anatomists, and not drudges. Under these conditions the finest spirit often permeates the entire staff, and for this the professor is largely responsible. After a professor has become well established, his desire for scientific work may wane. Those who have in them some spirit of altruism may continue to contribute to science, but the world, which is not inclined to believe in ideal motives, confounds this with self-seeking ambition. To the extent one's work is reflected in one's pupils and not in one's own publications, to that extent the motive certainly is altruistic. To carry out this ideal the large research wing of the institute exists and it is to be hoped that the altruistic spirit of Döllinger will continue to live in this anatomical palace for generations to come.

FRANKLIN P. MALL.













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